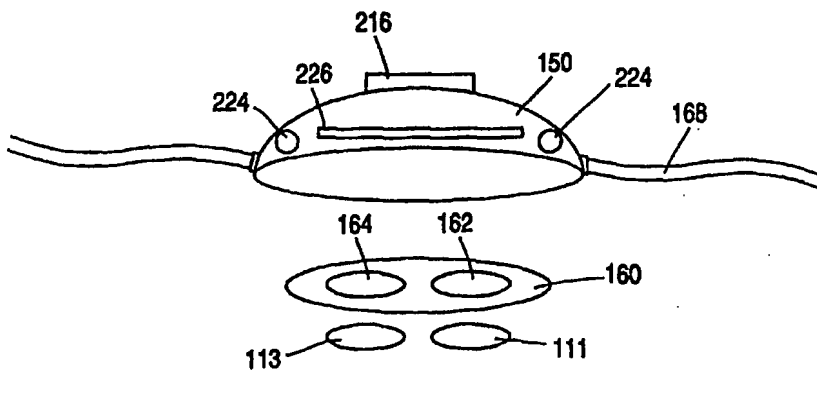




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(54) Title: IONTOPHORETIC SAMPLING DEVICE AND METHOD



## (57) Abstract

An iontophoresis apparatus for the transdermal monitoring of a target substance comprising: first and second collection reservoirs, the first collection reservoir comprising a first ionically conductive medium (111) and the second collection reservoir comprising a second ionically conductive medium (113); a first iontophoresis electrode (162) in contact with the first conductive medium (111) and a second iontophoresis electrode (164) in contact with the second conductive medium (113); a sensor for detecting the target substance contained within at least one conductive medium (111, 113); and an iontophoretic power source (224). The conductive medium (111, 113) is either an ionically conductive hydrogel or a wicking material containing an ionically conductive medium. The invention also includes a collection reservoir for use with an iontophoresis apparatus for the transdermal monitoring of a target substance, the collection reservoir comprising an ionically conductive hydrogel having a pH in the range of from about 4.0 to about 10.0 and an enzyme reactive with the target substance. The invention also includes a method for using the transdermal monitoring apparatus. In particular, the invention is a method for continuous *in vivo* monitoring of the blood glucose level of a patient comprising the following steps: (a) placing a collection reservoir on a collection site on a tissue surface of the patient; (b) applying electrical energy to the collection site to move glucose or a glucose metabolite into the collection reservoir; (c) analyzing the collection reservoir for concentration of glucose or glucose metabolite; (d) correlating the concentration determined in step (c) with blood glucose level; and (e) performing steps (a)-(d) substantially continuously.

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## IONTOPHORETIC SAMPLING DEVICE AND METHOD

BACKGROUND OF THE INVENTION

This invention relates generally to the  
5 transdermal monitoring of the concentration of substances  
in blood and, in particular, to the use of reverse  
iontophoresis or electroosmosis for the continuous  
transdermal monitoring of blood glucose.

Many diagnostic tests are performed on humans  
10 by evaluating the amount or existence of substances in  
the blood. One blood substance of interest is blood  
glucose. Typically, blood samples are removed from a  
subject by either using a syringe or by pricking the  
skin. The amount of blood drawn, of course, depends upon  
15 the amount of blood required for testing. Thereafter,  
the blood sample may be prepared and specifically tested  
for a variety of substances using techniques well known  
in the art.

Over the last few years methods of determining  
20 the concentration of blood glucose or other substances  
without drawing blood have been developed. For example,  
Stanley U.S. Patent No. 5,139,023 describes a transdermal  
glucose monitoring apparatus that uses a permeability  
enhancer, such as a natural bile salt, to facilitate  
25 transdermal movement of glucose along the concentration  
gradient between the higher glucose concentration in the  
interstitial fluid and the lower glucose concentration in  
the receiving medium. In some embodiments, the receiving  
medium is the aqueous portion of a hydrogel support  
30 adhered to the subject's skin. Stanley measured the  
glucose concentration within the hydrogel by removing the

hydrogel from the subject's skin, placing the hydrogel in water and letting the glucose diffuse out of the hydrogel into the water. The water was then analyzed for glucose concentration.

5                   Sembrowich U.S. Patent No. 5,036,861 describes a glucose monitor that collects the subject's sweat through a skin patch attached to the subject's wrist. Sembrowich describes the use of iontophoresis to transdermally introduce a gel into the subject's skin.  
10       The gel contains a cholinergic agent for stimulating the secretion mechanism of the eccrine sweat gland and agents that minimize or prevent loss of glucose from the sweat as it travels from the sweat gland to the skin patch. The Sembrowich device uses electrodes to measure the  
15       glucose level in the collected sweat by some unspecified method.

                  Schoendorfer U.S. Patent No. 5,076,273 describes a method and apparatus for determination of chemical species in body fluid. Sweat expressed from the  
20       subject's skin is collected in a patch adhered to the subject's skin. The patch concentrates the sweat in a binder layer by driving off a portion of the collected water. The collected analyte binds with a specific binding partner in the patch to present a visual  
25       indication of its presence in the patch.

                  Schroeder U.S. Patent No. 5,140,985 discloses a sweat-collecting device mounted on a subject. The device has an electrode-based glucose detection system that can give a qualitative indication of blood glucose  
30       concentration.

                  Glikfeld U.S. Patent No. 5,279,543 describes the use of iontophoresis to sample a substance through skin into a receptor chamber on the skin surface. In one embodiment, Glikfeld describes an in vitro device  
35       consisting of two gel electrodes attached to one side of

hairless mouse skin. Radiolabeled glucose is placed on the other side of the skin, and current is applied to the electrodes for a period of time. The electrodes are then analyzed for radioactivity content by conventional liquid  
5 scintillation counting. (See Glikfeld col. 7, line 51, to col. 8, line 1.) Glikfeld suggests that this sampling procedure can be combined with a specific glucose biosensor or glucose selective electrodes, but Glikfeld does not describe how that combination might be achieved.  
10 (See Glikfeld col. 9, lines 27-34.)

#### SUMMARY OF THE INVENTION

This invention improves on prior art transdermal monitoring methods and devices by providing  
15 an iontophoretic sampling device with an integrated sensor for monitoring the blood concentration of target substances or constituents noninvasively.

A general object of this invention is to provide a means of extracting substances in the blood  
20 through the skin using reverse iontophoresis or electroosmosis. In a preferred embodiment, one or more collection reservoirs are held in contact with the subject's skin. The reservoirs contain a conductive medium and are in turn in contact with electrodes and a  
25 means for providing electric potential or current between the collection reservoir site and another site on the subject's skin. The collection reservoirs are also in contact with a means for sensing and/or quantifying the concentration of a target substance.

30 This invention is particularly advantageous over drawing blood from a subject when extensive time-consuming sample preparation is required to perform a diagnostic test on whole blood. This invention is also advantageous for neonates (who have a small blood volume)  
35 and for subjects who must have frequent testing.

In a preferred embodiment, the collection reservoirs consist of an ionically conductive hydrogel or wicking material soaked with an ionically conductive medium. The advantage of using a hydrogel or wicking material as the extraction means is that by collecting the target substances into a semi-solid medium which is in contact with a target substance sensor, there is direct feedback to the subject of whether, or how much of, the target substance is in the blood. Also, because the target substances are extracted into a hydrogel or other medium of small volume, the concentration of the substance is higher than can be obtained by reverse iontophoretic extraction into a liquid solution, as in some prior art devices.

There are a number of different substances that can be sampled using the reverse iontophoresis/electro-osmosis techniques described herein. Charged (i.e., having a negative or positive ionic charge) substances will be extracted in the highest concentration. Uncharged substances will be extracted at lower, but still quantifiable, concentrations. An example of an uncharged substance is glucose. Obviously, there are many other substances in the body which would be of interest to sample and analyze. These include, but are not limited to, amino acids, enzyme substrates or products indicating a disease state or condition, other markers of disease states or conditions, drugs of abuse, therapeutic drugs, and electrolytes.

Different substances will be extracted at each electrode, as one electrode is positively charged and will attract negatively charged substances, and the other electrode is negatively charged and will attract positively charged substances. Uncharged substances may be extracted at either electrode, although they are typically extracted at higher concentrations at the

negatively charged electrode. Each ionically conductive medium in contact with the electrodes, therefore, may be analyzed for different substances.

The invention is particularly (although not exclusively) directed to a method for continuous *in vivo* monitoring of the blood glucose level of a patient comprising the following steps:

- (a) placing a collection reservoir on a collection site on a tissue surface of the patient;
- 10 (b) applying electrical energy to the collection site to move glucose or a glucose metabolite into the collection reservoir;
- (c) analyzing the collection reservoir for concentration of glucose or glucose metabolite;
- 15 (d) correlating the concentration determined in step (c) with blood glucose level; and
- (e) performing steps (a)-(d) substantially continuously.

The method correlates measured glucose levels with blood glucose levels over an extended period of time and tracks changes in blood glucose levels.

The invention is also particularly, but not exclusively, directed to a glucose monitor comprising:

first and second glucose collection reservoirs each containing glucose collection medium selected from a group consisting of water and saline solution;

a power supply having a positive connector and a negative connector;

conductors and a switch electrically connecting the first and second collection reservoirs to the power supply positive connector and the power supply negative connector, the switch having a first position in which the first collection reservoir is electrically connected to the positive connector and the second collection reservoir is electrically connected to the negative

connector and a second position in which the second collection reservoir is electrically connected to the positive connector and the first collection reservoir is electrically connected to the negative connector.

5 Preferably, the glucose monitor is one wherein the first and second collection reservoirs each further comprise an electrode in electrical communication with the collection medium and with the power supply through a conductor and the switch.

10 The invention is described in more detail below with reference to the drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

15 Figure 1A is a block diagram of a glucose monitoring device according to this invention.

Figure 1B shows a glucose monitoring device useful for practicing this invention in place on a patient.

20 Figure 1C is an exploded drawing of a collection reservoir assembly.

Figure 1D is a cross-sectional drawing of the electrode/collection reservoir assembly of Figure 1C.

25 Figure 2 is a graph of the results of a blood glucose monitoring of a fasting subject (1) using a standard protocol.

Figure 3 is a graph of the results of a blood glucose monitoring of a fasting subject (2) using a standard protocol.

30 Figure 4 is a graph of the results of a blood oral glucose tolerance monitoring of subject (1) as Figure 2 using a standard protocol.

Figure 5 is a graph of the results of a blood oral glucose tolerance monitoring of subject (1) as Figure 4 showing only the cathode.



Figure 6 is a graph of the results of a blood oral glucose tolerance monitoring of a subject (1) as Figure 5 at the cathode showing a 40 min lag time.

5 Figure 7 is a graph of the results of a blood glucose monitoring of a fasting subject (1) as in Figure 2 showing a drift in flux at the cathode.

Figure 8 is a graph of the results of a blood glucose monitoring of a fasting subject (1) as in Figure 7 showing a linear fit to the drift at the cathode in  
10 flux over 3 h.

Figure 9 is a graph of the results of a blood oral glucose tolerance monitoring of a subject (1) as in Figure 4 showing subtraction of linear drift as in Figure 8 from cathode flux.

15 Figure 10 is a graph of the results of a blood oral glucose tolerance monitoring of a subject (1) as in Figure 9, showing extracted glucose correlates with blood glucose with a 40 minute lag as in Figure 6.

Figure 11 is a graph of the results of an oral glucose tolerance monitoring of a subject (3) showing  
20 correlation at the anode using a standard protocol.

Figure 12 is a graph of the results of an oral glucose tolerance monitoring of a subject (3) as in Figure 11 showing a 20 min time lag.

25 Figure 13 is a graph of the results of an oral glucose tolerance monitoring of a subject (4) showing higher flux and better correlation at the anode using a standard protocol.

Figure 14 is a graph of the results of an oral glucose tolerance monitoring of a subject (4) as in  
30 Figure 13 showing a 20 min time lag.

Figure 15 is a graph of the results of an oral glucose tolerance monitoring of a subject (5) showing correlation at both the cathode and anode using a  
35 standard protocol.

Figure 16 is a graph of the results of an oral glucose tolerance monitoring of a subject (5) as Figure 15 showing a 20 min time lag.

5 Figure 17 is a graph of the results of a blood oral glucose tolerance monitoring experiment on subject (6), right arm, using a standard protocol.

Figure 18 is a graph of the results of a blood oral glucose tolerance monitoring experiment on subject (6), left arm, using an alternating protocol.

10 Figure 19 is a graph of the results of a blood oral glucose tolerance monitoring experiment on subject (7), right arm, using a standard protocol.

Figure 20 is a graph of the results of a blood oral glucose tolerance monitoring experiment on subject 15 (7), left arm, using an alternating protocol.

Figure 21 is a graph of the results of a fasting blood glucose monitoring experiment on subject (6) using an alternating protocol.

20 Figure 22 is a graph of the results of a fasting blood glucose monitoring experiment on subject (6) as Figure 21 showing only the cathode.

Figure 23 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 18, left arm, but with the drift adjusted for the cathode as 25 in Figure 22.

Figure 24 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 18 but with the time lag and drift adjusted for the cathode.

30 Figure 25 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 18 but with the drift adjusted for the anode as in Figure 21.

35 Figure 26 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 18 but with the time lag and drift adjusted for the anode.

Figure 27 is a graph of the results of a blood glucose monitoring of the same subject (7) as Figure 20, left arm, but with the time lag and drift adjusted for the cathode.

5           Figure 28 is a graph of the results of a blood glucose monitoring of the same subject (7) as Figure 20, left arm, but with the time lag and drift adjusted for the anode.

10           Figure 29 is a graph of the results of a blood glucose monitoring experiment using several cycles of direct current and several cycles of alternating polarity.

15           Figure 30 is a graph of the results of iontophoretic sampling of glucose compared to passive and wash controls.

            Figure 31 is a graph of the cumulative amount of glucose extracted passively from the skin plotted as a function of the square-root of time. Each line corresponds to a single subject.

20           Figure 32 is a graph of the results of glucose sampling at pH 8.2 using the standard method.

            Figure 33 is a graph of the results of glucose sampling at pH 8.2 using alternating polarity.

25           Figure 34A is a top view of an embodiment of the invention featuring a co-planar iontophoresis electrode and sensor electrodes.

            Figure 34B is a cross-sectional side view of the embodiment of the invention shown in Figure 34A.

30           Figure 35A is a top view of another embodiment of the invention in which the sensor electrodes are located on top of the iontophoresis electrode.

            Figure 35B is a cross-sectional side view of the embodiment of the invention shown in Figure 35A.

Figure 36A is a top view of an embodiment of the invention in which the sensor electrodes are located beneath the iontophoresis electrode.

Figure 36B is a side view of the embodiment of the invention shown in Figure 36A.

Figure 37A is a top view of an embodiment of the invention featuring an annular iontophoresis electrode surrounding the sensor electrodes.

Figure 37B is a side view of the embodiment shown in Figure 37A.

Figure 38 is an exploded view of the embodiment of Figure 37 and a strap-on housing.

Figure 39 is a side view showing an optical sensing scheme.

Figure 40 is a schematic diagram showing an iontophoretic collection system according to this invention.

Figure 41 is an exploded view of one embodiment of an iontophoretic collection system according to this invention.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

##### Definitions

Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in art to which this invention is directed. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of this invention, the preferred methods and materials are now described.

All patents and publications cited herein are incorporated herein by reference for the purpose of disclosing and describing information for which the patents and publications are cited in connection with.

The following terms are used throughout the application:

By "applying electrical energy to the collection site to move glucose or a glucose metabolite into the collection reservoir" is meant applying electrical energy of sufficient strength and duration to the tissue surface of a patient in order to transport the glucose or a glucose metabolite from beneath the tissue at a collection site on the surface of the tissue into a defined collection area. This includes the method known as iontophoresis.

"Iontophoresis" as used herein means a method for transporting positive or negative ions or even uncharged non-ionic substances across tissue through the application of electrical energy to the tissue. In conventional iontophoresis, a reservoir is provided at the tissue surface to serve as a source of material to be transported or as a collector of material transported.

"Reverse iontophoresis" refers to the movement of an ionically charged substance from the blood across the epithelial membrane and into a collection device under the influence of an applied electric potential. "Electroosmosis" refers to the movement of a charged or uncharged substance through a membrane into a collection device under the influence of an applied electric potential. "Reverse iontophoresis" and "electroosmosis" will be used interchangeably herein to refer to the movement of an ionically charged or uncharged substance across an epithelial membrane upon the application of an electric potential to the epithelial membrane through an ionically conductive medium.

As used herein "sampling" or "monitoring" as used herein means the extraction of a substance from an organism through the tissue. The tissue or skin can be natural or artificial tissue and can be of plant or

animal nature, such as natural or artificial skin, blood vessel tissue, intestinal tissue and the like. The term "artificial" as used herein means an aggregation of cells of monolayer thickness or greater which are grown or  
5 cultured in vivo or in vitro and which function as a tissue of an organism but are not actually derived, or excised, from a pre-existing source or host. The subject/patient or host organism can include warm-blooded animals and is particularly, a mammal, such as rats,  
10 mice, pigs, dogs, cats, cattle, sheep, horses and especially a human being. When the tissue is human skin, the tissue surface is the stratum corneum surface or mucosal tissue, such as in the oral, nasal or vaginal cavity.

15 "Epithelial layer" refers to a membrane surface, such as skin and mucosal membranes.

#### General Materials and Methods

The method and apparatus of this invention may  
20 be used to transdermally sample and quantify or qualify the level of a substance in a patient's blood. The method and apparatus will be initially described as used to transdermally sample and quantify or qualify glucose or a glucose metabolite. It should be understood that  
25 the method and apparatus of this invention may be used to transdermally sample and quantify or qualify other substances as well.

There is a need to sample and quantify or  
30 qualify glucose or a glucose metabolite in the blood, for example, for the purpose of diabetes diagnosis and treatment. This preferably needs to be done without the invasive withdrawal of blood by a needle into a collection reservoir/container. For maximum benefit, the sampling and quantification also need to be performed

substantially continuously and the measured glucose values need to track actual blood glucose levels.

Accordingly, the method of the invention is useful in continuously monitoring the levels of glucose or a glucose metabolite from the body. The method can also be used for the measurement of blood glucose levels in either a semi-continuous or a single measurement method. The method can be practiced by a device that provides electrodes or other means for applying electric current to the tissue at the collection site; one or more collection reservoirs or sampling chambers to receive the glucose; and a glucose concentration measurement system. A preferred embodiment of the method and apparatus are described in more detail below.

According to the method of this invention, a glucose collection reservoir is placed at a collection site on a tissue surface of the patient, for example, on the stratum corneum of the patient's skin or on the mucosal epithelium. Electrical energy is applied to the tissue at the collection site to move glucose or a glucose metabolite from the tissue into the collection reservoir. The collection reservoir is analyzed periodically to measure the glucose or glucose metabolite concentration therein, and this measured concentration value is correlated with the patient's blood glucose level. These steps are repeated to monitor the patient's blood glucose level substantially continuously and to track changes in the patient's blood glucose level.

In a preferred embodiment of the method, two electrodes provide the electrical energy to the tissue at the collection site or sites. Two collection reservoirs are provided, one at or near each electrode. The applied electrical current is preferably in the range of about 0.01 to about 2 mA/cm<sup>2</sup>. In applying the electrical energy, the polarity of the electrodes is alternated at a

rate in the range of about 0.1 Hz (one switch every 10 seconds) to about 1 switch every hour, so that each electrode is alternately a cathode or an anode. Glucose is collected in both reservoirs, but is most preferably measured in the reservoir acting as the cathode. In other words, the reservoir in which glucose concentration is monitored alternates as the polarity of the electrodes alternates.

The reversal of polarity during the glucose sampling method of the invention has advantages, including: (1) an unexpected enhancement of the correlation between blood and iontophoretically extracted samples under the alternating polarity method of the invention as compared to a non-alternating polarity protocol, (2) a particularly unexpected increase in the normalized flux rate, and (3) the avoidance of depletion of the AgCl plating on AgCl electrodes or pH variations which occur for non-alternating systems.

The polarity switching can be manual or automatic. The polarity switching can be of any frequency, especially at frequencies between about 1 cycle per 20 seconds to about 1 cycle per 4 hours, preferably between about 1 cycle per 20 seconds to about 1 cycle per 2 hours, more preferably between about 1 cycle per minute to about 1 cycle per 2 hours, or between about 1 cycle per 10 minutes to about 1 cycle per hour, and especially about 1 cycle per half hour. By cycle is meant one interval of current in each direction. The application of electrical energy in a given cycle may cease during glucose analysis of the collection medium.

In the preferred method, part or all of the glucose collection medium is extracted from the collection reservoir and analyzed for glucose concentration. One preferred analysis method is high



pressure liquid chromatography (HPLC), although other analysis methods may be used.

To provide useful information regarding blood glucose levels, the glucose concentrations detected in the collection medium must be correlated with the patient's blood glucose. Collection medium glucose is computed in flux terms, such as  $\text{nmoles/cm}^2 \cdot \text{hr}$ . This information must be translated into blood glucose values (expressed, e.g., in terms of  $\text{mg/dl}$ ) that correspond to the measured glucose flux.

The correlation between blood glucose concentration and measure glucose flux may be expressed in terms of four variables: the time lag between blood glucose concentration values and measured glucose flux values; linear drift over time of the measured glucose flux values; and the slope and intercept (i.e., the low-end blood glucose value below which no glucose flux is detected) of a line relating time lag and drift-corrected glucose flux data to blood glucose concentration. Correlation between measured glucose flux and actual blood glucose concentration is accomplished through a calibration procedure which relates blood glucose data and glucose flux data.

Time lag is defined as the constant time shift between blood glucose and glucose flux curves that achieves a best fit in curve shape along the time axis within predefined tolerances. The amount of time shift may be determined manually or automatically with the aid of a computer or other processor using known curve shape comparison algorithms. Time shift is illustrated in Figs. 5 & 6; Figs. 9 & 10; Figs. 11 & 12; Figs. 13 & 14; Figs. 15 & 16; Figs. 23 & 24; and Figs. 25 & 26.

Drift is defined as the linear change with time of the measured glucose flux for a given blood glucose concentration. Drift is computed by shifting the plotted

glucose flux curve with respect to the blood glucose concentration curve along the glucose flux axis by a constant multiple of the time variable to achieve a best fit. The value of the constant multiple may be computed  
5 in many different ways. For example, experimental data taken from a given subject at given times under "baseline" conditions (i.e., the patient is fasting and the blood glucose level is therefore constant) may be subtracted from measured glucose flux data point by point  
10 during the oral glucose tolerance test at the same times for the same patient.

As another example, a linear fit (obtained, e.g., from a least squares linear regression) of the baseline data for a given patient may be subtracted from  
15 experimental data obtained during the oral glucose tolerance test. This approach is illustrated in Figure 8 (baseline data), Figure 5 (experimental data without drift adjustment), Figure 9 (drift-adjusted experimental data) and Figure 10 (time lag and drift-adjusted  
20 experimental data).

Measured glucose flux values can also be corrected without first obtaining baseline values. One approach is to estimate the value of drift time constant, adjust for drift based on the estimate, and determine the  
25 correlation between drift-corrected measured values and measured blood glucose values. The estimated drift time constant can then be increased or decreased, and the correlation recalculated, until an acceptable tolerance between measure glucose flux values and blood glucose  
30 values is achieved.

The drift time constant may also be computed by measuring a different physiological parameter, such as the electrical resistance of the patient's skin (which may change with time in the same manner as the glucose  
35 flux value drift) or a parameter that remains relatively

constant over time, such as the pH of the patient's blood.

Finally, the slope and intercept of the relationship between the time and drift corrected values is computed using a linear best fit of the data. These steps may be performed by hand or by a suitably programmed processor or computer.

A certain amount of non-systemic glucose may be present in the stratum corneum prior to application of the glucose monitoring method and device as shown in Figures 2, 3 and 21. In the preferred embodiment, therefore, a time delay is provided between the start of the sampling and the time of analysis. This time delay may be on the order of 1 hour.

The method of this invention may be performed substantially continuously for an indefinite period of time. The method of the invention may also include the step of treating the patient in response to the determined (i.e., correlated) blood glucose level.

When the device is operated by alternating polarity, the collection reservoir/sampling chamber of the device of the invention can be in the form of (1) a single chamber with an electrode, which alternates polarity, with an indifferent counterelectrode which also alternates polarity, with the electrode in the sampling chamber acting as anode, cathode or both during the course of sampling, (2) two chambers in which the anode and cathode chambers are on alternate sites as the polarity alternates or (3) two or more chambers in which the polarity alternates during the treatment allowing for signal averaging between cathode and anode.

The collection reservoir/sampling chamber for receiving the sample of the target substance from the subject can be in the form of an adhesive patch enclosing

the electrode means. A wide variety of adhesives for patches are known in the iontophoresis art.

Figure 1A shows in block diagram form a continuous glucose monitor according to this invention.

5 The monitor includes a collection reservoir 200 containing a glucose collection medium. A pair of electrodes 202 and 204 connected to a power supply 206 having positive and negative connectors 207 and 208, respectively, via conductors 209 and 210 and a switch 212

10 provide current for transport of glucose from a tissue surface into reservoir 200. The switch 212 may be controlled manually or by an automatic controller 213. An analyzer 214 associated with the monitor measures the glucose concentration in glucose collection reservoir 200

15 and computes a glucose flux. A calibrator 216 correlates the computed glucose flux with blood glucose concentration.

Figures 1B-1D are drawings of a preferred device for practicing the method of this invention. As

20 shown in Figure 1B, two electrode/reservoir assemblies 110 are attached to collection sites 112 on a tissue surface 114 of a patient. The electrode/reservoir assemblies 110 are connected to a power supply 120.

Figure 1C is an exploded view of the

25 electrode/reservoir assembly 110. Element 101 is a release liner which is a protective layer for the adhesive of the skin contacting surface of the patch; element 102 is a sample port which can be a tube through which samples are removed; element 103 is the top layer, which can be a polymeric layer, which forms the upper

30 housing for the collection reservoir/sample chamber and makes it possible to view the condition of the electrode as the treatment progresses; element 104 is a foam layer coated on one side with an adhesive which prevents

35 contact of the electrode with the skin and serves as the

walls of the collection reservoir/sample chamber; element 105 is an electrode which serves as either the cathode (i.e., electron donor) or the anode (i.e., electron receiver) depending on the polarity of the electrodes; element 107 is a transfer adhesive layer which holds the electrode 105 and the sample port 102 in place; and element 108 is a protective layer positioned between the electrode 105 and the skin to prevent the electrode from contacting the skin.

As illustrated, layers 104 and 107 each comprise part of the housing of the collection reservoir and allow for the collection reservoir to be open to the skin.

The release liner 101 is a protective layer for the adhesive of the skin contacting surface of the patch. This liner can be any suitable flexible material conventionally known in the art. It should be strong enough not to be torn away by normal movement but easily removed by the application of some manual effort.

The sample port 102 can be a tube through which samples are removed.

The top layer 103 can be any flexible material conventionally known in the art, such as a polymeric layer, which forms the upper wall of the housing of the collection reservoir/sample chamber and is preferably transparent, which makes it possible to view the condition of the electrode as the treatment progresses. Any transparent polymeric material conventionally used as the top layer in transdermal patches can be used. Preferably, the top layer is made of polyester or preferably polyethylene.

The foam layer 104 is any medical grade foam layer (foam tape) coated on one side with an adhesive which prevents contact of the electrode with the skin and serves as the walls of the sample chamber. Any coated

foam layer (foam tape) which is conventionally used in transdermal patches which is compatible with the tissue and material to be sampled can be used, preferably the foam tape is a closed-cell polyolefin foam coated on one  
5 side with an acrylate adhesive, such as 3M # 9772.

The electrode 105 may be made of Ag/AgCl or any other electrode material. It is attached to the power source via electrical wires/leads.

The transfer adhesive layer 107 is a transfer  
10 adhesive layer which holds the electrode and the sample port in place. The adhesive can be any adhesive conventionally known in the art which can hold the layers together with the electrodes between. For example, the adhesive can be 3M # 1524, Medical Transfer Adhesive.

15 The protective layer 108 is positioned between the electrode 105 and the skin to prevent the electrode from contacting the skin. The protective layer is a polyurethane film coated on one side with an adhesive layer which keeps it in contact with the electrodes. Any  
20 conventional adhesive which is compatible with the skin can be used. For example, the protective layer is an Acutek tape (Flexcon-Deraflex NRU 100 clear H566 spec 50K-9 (nylon reinforced cast polyurethane coated with an acrylic adhesive)).

25 Figure 1D is a cross-section of an electrode/reservoir assembly 110 in place at the collection site 112. A collection reservoir/sampling chamber 116 is preferably filled with an electrically  
30 conducting medium/fluid. Any fluid which is capable of conducting electric current between the electrodes can be used. Suitable fluids include water, aqueous saline solutions and preferably aqueous buffered saline solutions having a pH in the range of from about 4 to  
35 about 9. A preferred fluid is an aqueous sodium bicarbonate buffered sodium chloride solution having a pH

of from about pH 7 to about pH 9. Obviously, the fluid is one that is inert to the material desired to be sampled (i.e., glucose) and compatible with skin. The collection medium provides an electrical circuit connection between electrode 105 and the tissue surface.

5 Instead of the collection reservoir described above, a collection matrix may be provided, as is known in the art. Also, electrodes in direct contact with the skin may be used in place of the electrodes immersed in conducted fluid as described above.

10 Preferably, power supply 120 comprises a means for reversing the polarity of the power source during the sampling, such as a manual or automatic programmable switch. One suitable power supply is the Iomed Phoresor2 iontophoretic power supply.

15 Also, when monitoring is conducted for more than 15 minutes, the data is self-consistent, with data at the cathode usually being the most efficient when not using the alternating polarity embodiment. One of skill in the art can recognize that there is variation between subjects or even for a given subject and for use of different iontophoretic device designs, surface area, current density, and the like as to background glucose, time lag and drift. These can be determined by standard methods and the correlation of the data adjusted for background glucose, time lag and drift for an individual and/or device.

20 Another apparatus according to the present invention consists of one or more reservoirs which contact the skin or other epithelial layer. While the present invention is intended to be used on any epithelial layer, some surfaces may be more desirable depending upon the target substance to be removed. For example, mucosal membrane has a lower barrier for non-ionized substances and also typically has a higher

25  
30  
35

concentration of blood vessels, making it more a more desirable substrate for the removal of uncharged substances.

The reservoirs each contain an ionically  
5 conductive medium. The conductive medium is preferably a hydrogel which contains ionic substances in an amount sufficient to produce high ionic conductivity. The composition of a gel according to a preferred embodiment of this invention is described in detail below. In an  
10 alternative embodiment, the ionically conductive medium could be a wicking material such as sponge, gauze pad, or other material that is soaked with an ionically conductive electrolyte solution, such as a 0.5% NaCl solution buffered to pH 8.0 with 0.5% sodium bicarbonate.

15 A first iontophoresis electrode contacts one reservoir (which is in contact with the subject's epithelial membrane) and a second iontophoresis electrode contacts either a second reservoir in contact with the epithelial membrane or some other ionically conductive  
20 medium in contact with the epithelial membrane. A power source provides an electric potential between the two electrodes to perform reverse iontophoresis on the epithelial membrane in a manner known in the art. Preferably, a sensor selected to sense the presence, and  
25 possibly the level, of a target substance within a reservoir is in contact with the reservoir. A display communicating with the sensor provides an indication of the presence and (possibly) the level of the target substance within the reservoir. If suitably calibrated,  
30 the display can indicate concentration of the target substance within the subject's blood. This monitoring can be performed substantially continuously, periodically, or on demand.

In Figure 34, two iontophoresis electrodes 16  
35 and 18, preferably made of Ag or Ag/AgCl, are in contact



with reservoirs 12 and 14. Each reservoir contains a conductive medium 11 and 13, each of which is in contact with the subject's epithelial membrane 15. In the preferred embodiment, the conductive medium of both reservoirs is a hydrogel. Alternatively, the conductive medium is an ionically conductive solution within a wicking material. A power source 24 provides an iontophoretic electric potential.

Also in contact with the conductive medium of both reservoirs are sensor electrode assemblies 28 and 30 to be used with a sensor apparatus (such as a potentiostat, not shown) to detect and/or quantify the extracted substances within the ionically conductive medium. Sensor electrode assemblies 28 and 30 may be connected to the sensor apparatus through conductors 29 and 31, respectively. Many types of electrode-based sensors and sensing methods are well-known in the art. For example, if the extracted substance of interest is glucose, a glucose sensor could be made by incorporating an enzyme specific to glucose in the ionically conductive medium and placing two or three electrodes in contact with the ionically conductive medium to detect the product of the reaction between glucose and the enzyme specific to glucose.

A housing 50 preferably surrounds both reservoirs and both sets of electrodes. Housing 50 is designed to hold the conductive medium in contact with the skin. The housing may also provide access for the electrical means and the sensor.

An alternative embodiment is shown in Figure 35. In this embodiment, the sensor electrode assemblies 28, 30 are disposed on the conductive medium of the reservoirs above the iontophoresis electrodes 16 and 18. The preferred conductive medium is once again a hydrogel, although the combination of a wicking material

and an ionically conductive medium may be used instead. In this embodiment, the iontophoresis electrodes are porous or made of a mesh-like material. The extracted substance can diffuse through the iontophoresis electrodes to the sensor electrode assemblies 28 and 30. The porous electrodes can be made of, for example, plastic, ceramic, metal mesh, or combinations thereof.

The pores of the electrodes should range in size from about 200  $\mu\text{m}$  to 1 mm. Optimally, the pores should be  $> 500 \mu\text{m}$  in size. Ideally, the number of pores is sufficient to give the electrodes a high porosity. Additionally, the surface of the pores may be hydrophilic. The hydrogel is drawn into the porous electrode via capillary action, thus creating ionic conductivity between the electrodes.

In an alternative embodiment shown in Figure 36, the sets of sensor electrode assemblies 28 and 30 are disposed within each reservoir's conductive medium beneath the iontophoresis electrodes 16 and 18. The sensor electrodes are therefore preferably porous (made, e.g., of a mesh-like material), as described above with respect to the iontophoresis electrodes shown in Figure 35. Once again, while the preferred conductive medium is a hydrogel, an ionically conductive medium in a wicking material may also be used.

In yet another alternative embodiment, the electrodes are arranged as shown in Figures 37A and 37B. Figures 37A and 37B show an iontophoretic collection apparatus 110 with two reservoirs 112 and 114 each having a conductive medium 111 and 113, preferably cylindrical hydrogel pads. Mounted on conductive media 111 and 113 are ring-shaped iontophoresis electrodes 116 and 118. Electrode 116 surrounds three sensor electrodes 128, 132 and 134. The sensor working electrode 128 is surrounded by the reference electrode 132 and counter electrode 134,

which are arc-shaped. A guard ring 140 runs between the sensor electrodes and the iontophoresis electrode 116 to minimize signal noise from the iontophoresis circuit. Conductors (not shown) run from the electrodes and the guard ring to outside power and ground sources and a sensor apparatus (not shown). Reservoir 114 has a similar electrode arrangement. This embodiment has the advantage of using most of the available space in the hydrogel pad while maximizing the size of the sensor working electrode, which in turn will maximize the signal from the sensor. As in the other embodiments, a wicking material soaked with an ionically conductive medium may be used in place of the hydrogel pads.

While Figure 37 shows cylindrical collection reservoirs and ring-shaped and arc-shaped electrodes, it should be understood that other reservoir and electrode geometries may be used as well without departing from the scope of this invention.

A suitable housing for the embodiment of Figure 37 is shown in exploded form in Figure 38. When assembled, the overall size and appearance of this embodiment is similar to a wristwatch.

The electrode assemblies of Figure 37 are represented in Figure 38 as elements 162 and 164 on an electrode board 160. In other words, electrode assembly 162 of Figure 38 corresponds to electrodes 118, 130, 136, 138 and 142 of Figure 37, and electrode assembly 164 of Figure 38 corresponds to electrodes 116, 128, 132, 134 and 140 of Figure 37. A connector 166 provides the electrical connection between the electrodes and the power source, ground source, and sensor apparatus.

In this embodiment, the reservoirs 112 and 114 are separable from their respective electrode assemblies 162 and 164. When assembled, however, the conductive media are in contact with the electrode assemblies.

Electrode board 160 and conductive media 111 and 113 are disposed within housing 150. In use, straps 168 hold the apparatus against the subject's skin 15 so that pads 111 and 113 are in contact with the skin. In addition, the power source (e.g., rechargeable or nonrechargeable batteries) may be disposed on the strap. In the preferred embodiment, the conductive media 111 and 113 are hydrogel pads, although the wicking material/ionically conductive medium combination may also be used.

In yet another alternative embodiment, an optical sensor can be employed as shown in Figure 39. The optical sensor need not be in direct contact with the ionically conductive medium. Alternatively, two sensors can be incorporated, one physically close to each iontophoresis electrode to measure the substance extracted at each electrode. These sensors may be selective for the same extracted substance or different extracted substances. The embodiment depicted in Figure 38 utilizes an optical colorimetric sensor 44 to detect and measure the extracted substance. The intensity of light generated by the light source 46, 48 is reflected from the conductive media (e.g., hydrogel pads) containing the color change chemistry. The light source 46, 48 are LEDs. Additionally, a baffle (not shown) may be placed between the light source and the detector to prevent light from reaching the detector directly.  $\text{TiO}_2$  (or other reflective material) can be added to the conductive media to increase the baseline reflectance.

In this embodiment, the inside of the housing 50 may be colored flat black to reduce the stray light reflections that may occur when the sensor employed is an optical sensor. Alternatively, the housing may be shaped and mirrored in order to act as a light-directing element to direct the maximum amount of LED intensity onto the

hydrogel and to collect the maximum amount of reflected light onto the detector for the Figure 38 embodiment discussed below.

5 In an embodiment where the conductive medium is a relatively transparent and translucent hydrogel, a double pass absorbance cell (not shown) may be created by placing a reflective material, such as  $\text{TiO}_2$  coated porous membrane or  $\text{Al}_2\text{O}_3$  porous membrane, on the skin side of the hydrogel. In this embodiment, the light from the LED  
10 would pass through the hydrogel, reflect, and pass through the hydrogel again.

In any of the hydrogel or wicking material  
embodiments, the conductive medium is preferably attached to the epithelial membrane using an adhesive. The  
15 adhesive properties can be inherent in the conductive medium (as is the case for some hydrogels) or added to the conductive medium through a separate adhesive element. If the conductive medium is not sufficiently adhesive, a separate means of holding the conductive  
20 medium in contact with the skin (such as a strap) may be used.

In the embodiments described above, the reservoirs are physically separated within housing.  
50. If necessary, a non-porous, electrically insulating  
25 element can be placed between the two ionically conductive media to prevent contact.

The hydrogel used in this invention must (1) be ionically conductive, (2) provide a hospitable environment for the sensor enzyme, and (3) not interfere  
30 with sensor operation. An example of the preparation of a suitable ionically conductive and biosensing hydrogel for use in monitoring glucose with the embodiments discussed above is as follows.  $\text{NaCl}$  and  $\text{NaHCO}_3$  (food grade) are dissolved in water to make a solution in the  
35 range of about 0.1% to about 1.0%, preferably about 0.5%.

Cross-linked acrylic acid polymer (preferably, Carbopol 934 PNF, available from B.F. Goodrich) is suspended into the  $\text{NaCl} \cdot \text{NaHCO}_3 \cdot \text{H}_2\text{O}$  solution. The pH of the resulting solution is tested with an Oaktron pH Tester 1, and then  
5 adjusted to from about pH 4.0 to about pH 10.0, preferably from about pH 6.0 to about pH 8.0, using food grade sodium hydroxide (available from Malinckrodt). The actual pH desired depends on the enzyme to be used for the biosensor; the ranges provided here are for glucose  
10 detection. Glucose oxidase in a range from about 50 I.U. to about 100,000 I.U., preferably from about 300 I.U. to about 5000 I.U., is added to the pH-adjusted  $\text{NaCl} \cdot \text{H}_2\text{O} \cdot \text{Carbopol}$  solution. The actual amount of glucose oxidase depends on the desired shelf life of the product,  
15 the intended use life of the product, and the stability of the enzyme during storage.

Additional substances may be added to the solution. These include, but are not limited to, for example, other salts, other buffers, tackifiers,  
20 biocides, preservatives and enzyme stabilizers.

In an alternative embodiment, it is possible to store the conductive media in a dry form. Dry forms include, but are not limited to, a dehydrated gel, or a sponge containing the components necessary to allow  
25 proper operation of the device. Water, or electrolyte solution could then be added to the medium before the conductive media are attached to the skin, thus rehydrating the medium. An advantage of dry storage is that it allows for greater stability of the components of  
30 the medium during storage and also allows for longer shelf life.

In practice, an electric potential (either direct current or a more complex waveform) is applied between the two iontophoresis electrodes 16 and 18 such  
35 that current flows from the first electrode through the

first conductive medium into the skin, and back out from the skin through the second conductive medium to the second electrode. This current flow extracts substances through the skin into one or both reservoirs 12 and/or 14 through the process of reverse iontophoresis or electroosmosis. The electric potential may be applied as described in copending U.S. Patent Application S.N. 08/265,048, "Method of Continuous Iontophoretic Measurement of Chemical Species in Body Fluids," filed June 24, 1994, the disclosure of which is incorporated herein by reference.

As an example, to extract glucose, the applied electrical current density is preferably in the range of about 0.01 to about 2 mA/cm<sup>2</sup>. In a preferred embodiment, in order to facilitate the extraction of glucose, electrical energy is applied to the electrodes 16, 18, and the polarity of the electrodes is alternated at a rate in the range of about 0.1 Hz (one switch every 10 seconds) to about 1 switch every hour, so that each electrode is alternately a cathode or an anode.

The polarity switching can be manual or automatic. The polarity switching can be of any frequency, especially at frequencies between about 1 cycle per 20 seconds to about 1 cycle per 4 hours, preferably between about 1 cycle per 20 seconds to about 1 cycle per 2 hours, more preferably between about 1 cycle per minute to about 1 cycle per 2 hours, or between about 1 cycle per 10 minutes to about 1 cycle per hour, and especially about 1 cycle per half hour. By cycle is meant one interval of current in each direction. The application of electrical energy in a given cycle may cease during glucose analysis of the collection medium.

As discussed above, the iontophoresis electrodes are connected to a power source and the sensor electrodes are connected to a sensor apparatus. Figure

40 is a circuit block diagram showing the elements of an iontophoretic monitoring system according to a preferred embodiment. Incorporated into the collection reservoir portion 204 of the system are the iontophoresis electrodes 208 and the sensor electrodes 210. These electrodes are in contact with the conductive medium of each reservoir. Also included is an optional temperature sensing element 206 (such as a thermistor) which monitors the temperature at the collection reservoir to enable temperature correction of the sensor signal, if necessary.

The power and control portion 202 of the system includes a controller 212 (such as a microprocessor), a power supply 214, and a display (or other output) 216. The display may be used, for example, to allow patients to scroll through their present and previous analyte (e.g., glucose) level readings and to alert patients to fluctuations in their levels. The power supply 214 is shown as having two parts, one for the iontophoresis function and one for the sensing function. The two parts may not be necessary, of course, depending on the approach chosen for the iontophoresis and sensing functions. Also included in the system are an optional alarm 218, optional input/output ports 220 and a memory 222 to store and recall data, such as, e.g., periodic glucose readings.

In one embodiment, the collection reservoir portion 204 of the system is contained in one housing (such as the housing 150 shown in Figure 38), and the power and control portion 202 of the system is contained in a separate housing. Electrical conductors connect the two housings to carry power, control signals and data.

An alternative embodiment is shown in Figure 41. In this embodiment, the power and control elements are contained within the same housing as the collection



reservoir portion of the system. A display 216 is provided at the top of the housing 150. Batteries 224 are part of the power supply. The microprocessor, memory and other circuit components are mounted on PC board 226  
5 disposed within housing 150.

A process of using the iontophoretic collection system of this invention to monitor blood glucose concentration is as follows. This example uses two hydrogel pads made substantially as described above for  
10 the conductive media of the collection reservoirs, the electrode assembly shown in Figure 37 and the housing arrangement shown in Figures 38 and 41.

The monitoring process begins by placing two hydrogel pads in contact with the electrode assembly  
15 within the housing. The housing is then strapped to the subject so that the pads are in contact with the subject's skin. Power is then provided to the iontophoresis electrodes to begin the collection process.

As glucose travels across the subject's skin  
20 into the hydrogel pads, the glucose reacts with the glucose oxidase within the pads to generate hydrogen peroxide. The presence of hydrogen peroxide generates a current at the sensor working electrode that is proportional to the concentration of the hydrogen  
25 peroxide within the pad. The current provides a signal that is interpreted by the system controller to provide a glucose concentration value for display. This current may be correlated with the subject's blood glucose concentration so that the system displays the subject's  
30 actual blood glucose concentration as measured by the iontophoretic collection system. For example, the system can be calibrated to the subject's actual blood glucose concentration by sampling the subject's blood through the standard pin prick technique, analyzing the blood glucose  
35 (using, e.g., a blood glucose monitor such as One Touch

Basic, LifeScan, Milpitas, CA), and correlating the glucose concentration measured in the blood with the iontophoretically extracted glucose concentration in the reservoir. The monitoring can be performed substantially  
5 continuously, periodically, or on demand.

The hydrogel pads contain an amount of glucose oxidase sufficient to provide a predetermined monitoring period. After that period expires, the user should replace the used hydrogel pads for new ones. This  
10 invention facilitates that operation by providing a design in which the hydrogel pads are easily separable from the rest of the iontophoretic collection system.

In an alternative embodiment, the system can be provided with a system that determines whether a hydrogel  
15 pad is operational or not. For example, a new pad contains virtually no hydrogen peroxide (e.g., below 80  $\mu\text{M}$ ) prior to activation of the iontophoretic collection process. If the system detects the presence of hydrogen peroxide above a threshold amount upon start-up (i.e.,  
20 before the application of any iontophoretic potential to the iontophoresis electrodes), it can warn the user (e.g., by activating an alarm) that the pad should be replaced with a new pad.

In another alternative embodiment, the two  
25 hydrogel pads can be formed as a single element, such as by incorporating an insulator to separate the hydrogel into two functional elements.

Other modifications will be apparent to those skilled in the art.

30

#### EXAMPLES

The following examples are provided to illustrate the invention but should not be regarded as limiting it in any way.

35

Example 1

A continuous iontophoretic extraction was conducted on subjects who fasted 12 hours overnight prior to the start of the experiment and during the experiment. In these experiments, two adhesive sampling patches, each with Ag or AgCl electrodes inserted into the aqueous sampling chamber of the patches were applied to the forearm. The iontophoretic patches had a collection surface area of 2.85 cm<sup>2</sup> per patch and a collection reservoir volume of 0.4 mL per patch. The chambers were filled with 0.45% sodium chloride in water, U.S.P. For the direct current or DC protocol (the standard protocol), an electric current of density 0.32 mA/cm<sup>2</sup> was applied for 15 min, followed by a 5 min rest interval in which the saline was removed for analysis and then replaced with fresh saline. During the 5 min rest period, blood glucose was measured by a standard finger prick test (One Touch Basic, LifeScan, Milpitas, CA) method for comparison to iontophoretically extracted glucose. The 15 min iontophoresis and 5 min rest/sampling procedure was repeated for 3 to 5 h. The iontophoretically extracted samples were analyzed by HPLC.

For the passive controls, the same procedures were used except that no current was applied.

The results of these experiments are set forth in Figures 2 and 3. In each figure, blood glucose concentration in mg/dL is given versus time in minutes. The "x" is for blood glucose, solid triangle is for iontophoretic glucose at the anode, the solid square is for iontophoretic glucose at the cathode and the open circle for passive glucose.

The data in Figures 2-3 demonstrated that (1) (1) continuous extraction washed out interference from glucose in the skin (stratum corneum, epidermis, or

dermis), (2) the steady state was reached in less than one hour, (3) there was a drift in glucose flux, especially at the cathode and (4) cathode flux was greater than anode flux which was greater than passive flux.

### Example 2

An oral glucose tolerance test was conducted on non-diabetic subject (1) who had fasted prior to the experiment for 12 h overnight. One hour into the test, 75 gm of glucose was administered orally. Blood glucose levels increased from 100 to 180 mg/dL. Iontophoresis was administered and glucose was monitored as described in Example 1, using the standard DC protocol. The results of the test is set forth in Figures 4-10.

Figure 4 shows the results of the experiment. In each figure, blood glucose concentration in mg/dL is given versus time in minutes. The "x" is for blood glucose, solid triangle is for iontophoretic glucose at the anode, the solid square is for iontophoretic glucose at the cathode and the open circle for passive glucose. Figures 5 shows the same data as in Figure 4, but with only data for the cathode (the best response) shown. Figure 6 shows the same data as in Figure 5, but with the iontophoretically extracted glucose "shifted" 40 minutes compared to the blood glucose, illustrating a 40 minute time lag. In Figure 6, it is seen that the extracted glucose flux was not completely correlated to blood glucose, even with the time lag. The flux was relatively lower compared to the blood glucose at the start of the experiment and relatively higher than the blood glucose at the end of the experiment. Figure 7 shows that same data as Figure 2, but with only the data for the cathode shown. Figure 8 shows a linear fit to the data in Figure 7. The data in Figure 8 excluded the extraction samples

obtained before one hour into the extraction to eliminate the interference from glucose in the skin prior to the start of the experiment. Figure 9 shows the same data as Figure 5, but with the equation for the linear fit of Figure 8 subtracted from the data of Figure 5 to adjust for drift of glucose flux values over time. Figure 10 shows the data of Figure 6, adjusted for drift. Figure 10 demonstrates that there was a strong correlation between lag adjusted and drift adjusted iontophoretically extracted glucose and blood glucose.

Overall, the data in Figures 4-10 demonstrated that (a) there was correlation between glucose extraction at the cathode and blood glucose, (b) cathode flux was greater than anode flux which was greater than passive flux, (c) there was a time lag of about 40 min between changes in blood glucose and changes in extracted glucose, and (d) there was linear drift that was independent of blood glucose.

### 20 Example 3

Oral glucose tolerance tests were conducted on subjects (3), (4), and (5) following procedures similar to those described in Example 2 above. Results of these tests are set forth in Figures 11-16. In each Figure, blood glucose concentration in mg/dL is given versus time in minutes. The "x" is for blood glucose, solid triangle is for iontophoretic glucose at the anode, and the solid square is for iontophoretic glucose at the cathode.

Figures 11 and 12 are for subject (3). The data demonstrates that (a) there was a slight correlation between blood glucose and iontophoretically extracted glucose at the cathode, but there was much better correlation at the anode, (b) cathode flux was greater than anode flux, (c) anode flux tracked blood glucose with a slight drift and a time lag of 20 minutes.

Figures 13 and 14 are for subject (4). The data demonstrates that (a) there was a slight correlation between blood glucose and iontophoretically extracted glucose at the cathode, but there was much better  
5 correlation at the anode, (b) unlike the other subjects, anode flux was greater than cathode flux, and (c) anode flux tracked blood glucose with a slight drift and a time lag of 20 minutes.

Figures 15 and 16 are for subject (5). The  
10 data demonstrates that (a) there was a correlation between blood glucose and iontophoretically extracted glucose at both the anode and the cathode, (b) cathode flux was greater than anode flux, (c) both cathode and anode fluxes tracked blood glucose with a slight drift  
15 and a time lag of 20 minutes.

#### Example 4

Blood glucose monitoring experiments were performed in which a comparison was made between a  
20 standard iontophoretic protocol consisting of direct current between an anode site and a cathode site versus an alternating polarity iontophoretic protocol of the invention in which the electrodes act as both the anode and the cathode during the course of the extraction.

25 The alternating polarity protocol method of this invention was identical to the standard protocol, except that the polarity of the power supply was switched during each 5 min rest period. In this way, the cathode chamber of one 15 min period became the anode chamber of  
30 the next 15 min period, and vice versa.

Oral glucose tolerance tests were conducted on subjects (6) and (7), following procedures similar to those described in Examples 2 and 3 above. Results of these experiments are shown in Figures 17-20. These  
35 figures are graphs comparing the results of the standard

protocol and the alternating polarity method of the invention performed simultaneously on the same subject.

Figures 17 and 19 show the standard (DC) method which was performed on the right arm of subjects (6) and (7), respectively, and Figures 18 and 20 show alternating polarity protocol method of the invention, which was performed on the left arm of subjects (6) and (7), respectively. In each figures, blood glucose concentration in mg/mL is given versus time in minutes. The "x" is for blood glucose, the solid triangle is for iontophoretic glucose at the anode, and the solid square is for iontophoretic glucose at the cathode.

As can be seen by comparing the Figures 17 and 19 with Figures 18 to 20, respectively, the alternating polarity method of the invention gave more response and better correlation, particularly for the anode response.

The above results demonstrate the improvement in sampling profiles from alternating polarity, or the use of a single sampling electrode that acts, alternatively, as anode and cathode or even both during a cycling period during the course of the extraction, as compared to the direct current standard (DC) mode of iontophoresis.

The following are results of tests in which the data is correlated for time lag and/or drift as discussed below.

A continuous iontophoretic extraction was conducted on subject (6) as in Figure 18, but in a protocol in which the subject had fasted 12 hours overnight prior to the start of the experiment and during the experiment (no oral glucose tolerance test). The results are shown in Figures 21 and 22. Figures 21 and 22 demonstrate that there was a drift in the anode and cathode fluxes for subject (6).

Figure 23 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 18, but with the flux values for the cathode in Figure 22 subtracted from the flux values for the cathode of  
5 Figure 18.

Figure 24 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 23, but with the cathode flux adjusted for both a 20 minute time lag and the drift, as in Figure 22.

10 Figure 25 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 18, but with the flux values for the anode in Figure 21 subtracted from the flux values for the anode in Figure 18.

15 Figure 26 is a graph of the results of a blood glucose monitoring of the same subject (6) as Figure 25, but with the anode flux adjusted for both a 40 minute time lag and the drift, as in Figure 25.

20 Figure 27 is a graph of the results of a blood glucose monitoring of the same subject (7) as Figure 20 under the alternating polarity protocol, but with the cathode flux adjusted for time lag and a linear drift.

25 Figure 28 is a graph of the results of a blood glucose monitoring of the same subject (7) as Figure 20 under the alternating polarity protocol, but with the anode flux adjusted for time lag and a linear drift.

In total 12 normal subjects were tested. The results are summarized in Table 1 below.



Table 1

	Direct Current		Alternating Polarity	
	<u>Cathode</u>	<u>Anode</u>	<u>Cathode</u>	<u>Anode</u>
Good tracking	0	5*	7	4
5 Oscillation	0	0	5	6
Long Lag (>60 min)	5	1	0	2
No response	7	6	0	0
TOTAL	12	12	12	12

10 Good = tracking with drift =  $r^2 \geq 0.5$ , lag  $\leq 40$  min  
 Long lag =  $r^2 \geq 0.5$ , lag  $\geq 60$  min  
 Oscillation = qualitative tracking with oscillation (lag  $\leq 40$  min)  
 No response =  $r^2 \leq 0.5$   
 15 \*Anode tracking, but low magnitude of flux

The data demonstrates that there was a lower incidence of non-response and long time lag with the alternating polarity embodiment of iontophoresis administration.

#### Example 5

Saline was injected into each of two adhesive sampling patches, each containing an electrode. A current of 0.32 mA/cm<sup>2</sup> was applied through the electrodes for 15 minutes. The saline was then removed, a blood glucose finger prick measurement was made, and fresh saline was reinjected during a 5 minute rest period. The procedure was repeated 15 times for a total of 5 hours.

30 In this example, JK 1-17-94, the current was applied DC for 8 sample periods. On the 9th sample period, the current was switched to an alternating polarity protocol of a switch every 15 minutes. The results are set forth in Figure 29. There is a sharp and dramatic increase in flux as the protocol was switched from direct current to alternating polarity.

Example 6

Glucose monitoring was conducted on the ventral forearm of subjects. The collection reservoir was a thin, flexible, plastic device of area  $10 \text{ cm}^2$ , holding a volume of 0.55 mL. The relatively large surface area-to-volume ratio provided reasonable extraction efficiency. The collection reservoir held 0.1 M sodium chloride, and housed Ag/AgCl electrodes. Iontophoretic current was delivered to the electrodes from a custom built, computer-controlled power supply. Sampling was conducted at  $0.25 \text{ mA/cm}^2$  to avoid unnecessary sensation when the power supply was activated the current was "ramped" to its final constant value over a period of about 30 sec. Current was passed for periods of between 5 and 60 min. Prior to commencement of an experiment, the skin sites were left in contact with sodium chloride for 60 sec. This solution (the "wash") was then removed for analysis and replaced before activating current flow. Several control measurements (no-current) were also made. The most extensive of these involved repeated filling, emptying and replacement of the collection reservoir solutions as a function of time. This allowed determination of the passive extraction of cutaneous glucose. Analysis of glucose utilized a Dionex chromatographic system with pulsed amperometric detection. A 50 uL aliquot was used for each assay. The detection limit was 0.6 uM. Results of these experiments are set forth in Figures 30-31.

Figure 30 is the results of iontophoretic sampling of glucose at the cathode and anode compared to passive and wash controls.

Figure 31 is a graph of the cumulative amount of glucose extracted passively from the skin plotted as a function of the square-root of time. Each line corresponds to a single subject.

Example 7

Two iontophoretic extractions using a modified buffer solution were performed simultaneously on the same subject (A) as in Figures 17 and 18, one extraction using the standard (DC) protocol on the right arm and the other using the alternating polarity protocol on the left arm. Iontophoresis was administered and glucose was monitored as described in Example 4 above, with the following modification. Sodium bicarbonate buffer solution, 5% U.S.P., pH 7-9, mixed in a 1:9 ratio with 0.45% sodium chloride U.S.P. was used as the collector solution instead of 0.45% sodium chloride. The results are set forth in Figures 32 and 33.

Figure 32 demonstrated the dramatic enhancement in flux at the cathode for a direct current protocol using pH 8.2 sodium bicarbonate buffered saline solution compared to Figure 17 for the same subject (6) using 0.45% sodium chloride solution.

Figure 33 demonstrated the dramatic enhancement of flux at the cathode for the alternating polarity protocol using pH 8.2 sodium bicarbonate buffered saline solution compared to Figure 18 for the same subject (6) using 0.45% sodium chloride solution.

Further, a comparison of Figures 32 and 33 demonstrated that there was enhanced anode extraction for the sodium bicarbonate solution in the alternating polarity method of the direct current method.

What is claimed is:

1. An iontophoresis apparatus for the transdermal monitoring of a target substance comprising:  
5 a collection reservoir, the collection reservoir comprising either an ionically conductive hydrogel or a wicking material and an ionically conductive solution;  
first and second iontophoresis electrodes;  
10 a sensor for the target substance; and  
an iontophoretic power source.
2. The apparatus of claim 1 wherein the reservoir comprises an ionically conductive hydrogel.  
15
3. The apparatus of claim 1 wherein the reservoir comprises a wicking material and an ionically conductive solution.
- 20 4. The apparatus of claim 1 further comprising a second reservoir, the second reservoir comprising either a second ionically conductive hydrogel or wicking material and an ionically conductive solution.
- 25 5. The apparatus of claim 4 further comprising a housing and means for separably holding the first and second reservoirs within the housing in contact with the first and second iontophoresis electrodes, respectively.  
30
6. The apparatus of claim 1 wherein the sensor comprises a sensor electrode assembly.
7. The apparatus of claim 1 wherein the  
35 sensor comprises a plurality of sensor electrodes, the

sensor electrodes and the first iontophoresis electrode being disposed in substantially the same plane.

5           8. The apparatus of claim 7 wherein the reservoir has an electrode contact surface, the sensor electrodes and the first iontophoresis electrode being adapted to contact the electrode contact surface.

10           9. The apparatus of claim 6 wherein the sensor comprises an enzyme within the reservoir.

15           10. The apparatus of claim 6 further comprising a display for displaying target substance information.

20           11. An iontophoresis apparatus for the transdermal monitoring of a target substance comprising:  
            first and second collection reservoirs, the first collection reservoir comprising a first ionically conductive hydrogel and the second collection reservoir comprising a second ionically conductive hydrogel;  
            a first iontophoresis electrode in contact with the first hydrogel and a second iontophoresis electrode in contact with the second hydrogel;  
25           a sensor for detecting the target substance contained within at least one hydrogel; and  
            an iontophoretic power source.

30           12. The apparatus of claim 11 wherein the sensor comprises a first sensor electrode assembly in contact with the first hydrogel and a second sensor electrode assembly in contact with the second hydrogel.

35           13. The apparatus of claim 12 further comprising a housing containing the collection

reservoirs, the iontophoresis electrodes, the sensor electrode assemblies and the power source.

14. The apparatus of claim 12 further  
5 comprising means for separably holding the first and second hydrogels in contact with the iontophoresis electrodes and the sensor electrode assemblies.

15. The apparatus of claim 14 wherein the  
10 first and second hydrogels each have an electrode contact surface, the sensor electrode assemblies and the first iontophoresis electrode being adapted to contact their respective electrode contact surface.

16. The apparatus of claim 15 wherein the  
15 means for separably holding comprises an electrode board in which the iontophoresis electrodes and the sensor electrode assemblies are disposed.

17. The apparatus of claim 11 further  
20 comprising a display for displaying target substance information.

18. The apparatus of claim 17 further  
25 comprising a housing containing the collection reservoirs, the iontophoresis electrodes, the sensor electrode assemblies, the power source and the display.

19. A collection reservoir for use with an  
30 iontophoresis apparatus for the transdermal monitoring of a target substance, the collection reservoir comprising an ionically conductive hydrogel having a pH in the range of from about 4.0 to about 10.0 and an enzyme reactive with the target substance.

20. The collection reservoir of claim 19 wherein the enzyme is glucose oxidase.

21. The collection reservoir of claim 20 wherein the hydrogel has a hydrogen peroxide concentration less than 80  $\mu$ M.

22. The collection reservoir of claim 19 wherein the hydrogel further comprises an electrode contact surface adapted to contact an iontophoresis electrode.

23. The collection reservoir of claim 22 wherein the electrode contact surface is further adapted to contact a sensor electrode.

24. The collection reservoir of claim 23 wherein the hydrogel has a substantially cylindrical shape, the electrode contact surface being a circular surface at one end of the hydrogel cylinder.

25. A method for the transdermal monitoring of a target substance comprising the following steps:

providing a transdermal monitoring apparatus comprising first and second collection reservoirs, a first iontophoresis electrode in contact with the first reservoir and a second iontophoresis electrode in contact with the second reservoir, a sensor for detecting the target substance contained within at least one reservoir, and an iontophoretic power source;

applying a surface of each of the first and second reservoirs to an epithelial membrane of a subject;

applying an iontophoretic electric potential across the first and second iontophoresis electrodes;

monitoring at least one of the collection  
reservoirs for the target substance; and  
replacing at least one of the first and second  
collection reservoirs with a new reservoir.

5

26. The method of claim 25 wherein the  
monitoring step is performed substantially continuously.

10

27. The method of claim 25 wherein the  
monitoring step is performed periodically.

15

28. The method of claim 25 further comprising  
the step of displaying a concentration of the target  
substance.

20

29. The method of claim 28 wherein the  
concentration displayed in the displaying step is  
correlated with concentration of the target substance  
within the subject's blood.

25

30. The method of claim 25 wherein the target  
substance is glucose, the monitoring step comprising the  
step of detecting a current within a collection  
reservoir.

30

31. The method of claim 30 further comprising  
the step of detecting a current within a collection  
reservoir prior to the step of applying an iontophoretic  
electric potential and determining whether the current  
exceeds a threshold amount.

32. A method for continuous *in vivo* monitoring  
of a blood glucose level of a patient comprising the  
following steps:



(a) placing a collection reservoir on a collection site on a tissue surface of the patient;

(b) applying electrical energy to the collection site to move glucose or a glucose metabolite into the collection reservoir;

(c) analyzing the collection reservoir for concentration of glucose or glucose metabolite;

(d) correlating the concentration determined in step (c) with blood glucose level; and

(e) performing steps (a)-(d) substantially continuously.

33. The method of claim 32 wherein the tissue surface is a stratum corneum surface of the patient's skin.

34. The method of claim 32 in which the collection reservoir comprises a chamber containing a collection medium and having at least one opening permitting contact between the collection medium and the tissue surface.

35. The method of claim 34 in which the collection medium is electrically conductive and the collection reservoir further comprises an electrode attached to a power supply and in electrical communication with the collection medium, the applying step comprising supplying electrical current to the electrode.

36. The method of claim 32 wherein the collection reservoir is a first collection reservoir at a first collection site, the method further comprising the step of placing a second collection reservoir at a second collection site on the tissue surface, the applying step

comprising the step of applying electrical energy to the first and second collection sites to move glucose or a glucose metabolite into the first and second collection reservoirs, the analyzing step comprising analyzing the first and second reservoirs for concentration of glucose or glucose metabolite.

37. The method of claim 36 in which the applying step further comprises alternately applying a voltage between the collection sites in a first polarity and in a second polarity opposite to the first polarity.

38. The method of claim 37 in which the analyzing step further comprises alternately analyzing the first and second collection reservoirs after application of the voltage in the first polarity.

39. The method of claim 32 wherein the applying step is performed by supplying electrical energy to an electrode integral with the collection reservoir.

40. The method of claim 32 in which the step of applying electrical energy comprises iontophoresis.

41. The method of claim 32 in which the step of applying electrical energy comprises applying a current in the range of 0.1 to 2 mA/cm<sup>2</sup>.

42. The method of claim 32 in which the electrical energy is direct current.

43. The method of claim 32 in which the step of applying electrical energy comprises the step of ceasing the application of electrical energy during the analyzing step.

44. The method of claim 32 in which the analyzing step comprises pulsed amperometric detection:

5 45. The method of claim 32 in which the analyzing step comprises high performance liquid chromatography (HPLC).

10 46. The method of claim 32 wherein the tissue surface is a stratum corneum surface of the patient's skin, the analysis step comprising the step of delaying the time between the applying step and the analyzing step to allow for depletion of non-systemic glucose in the stratum corneum.

15 47. The method of claim 46 wherein the delaying step comprises delaying performance of the analyzing step for approximately one hour after commencing the analyzing step.

20 48. The method of claim 32 wherein the collection reservoir contains a collection medium, the analyzing step comprising the step of extracting at least a portion of the collection medium from the collection reservoir.

25 49. The method of claim 3 further comprising the step of providing correlation data between collection reservoir glucose level and patient blood glucose level.

30 50. The method of claim 49 wherein the correlating step comprises calculating glucose flux from the collection site into the collection reservoir.

51. The method of claim 50 wherein the correlating step also comprises compensating for time lag between blood glucose level and glucose flux.

5 52. The method of claim 51 wherein the correlating step also comprises compensating for glucose flux drift.

10 53. The method of claim 32 further comprising the step of treating the patient in response to blood glucose level determined in step (d).

15 54. The method of claim 32 wherein the application of electrical energy is by iontophoresis conducted with alternating polarity.

55. A glucose monitor comprising:  
first and second glucose collection reservoirs each containing glucose collection medium selected from a group consisting of water and saline solution;  
20 a power supply having a positive connector and a negative connector;  
conductors and a switch electrically connecting the first and second collection reservoirs to the power supply positive connector and the power supply negative connector, the switch having a first position in which the first collection reservoir is electrically connected to the positive connector and the second collection reservoir is electrically connected to the negative  
25 connector and a second position in which the second collection reservoir is electrically connected to the positive connector and the first collection reservoir is electrically connected to the negative connector.  
30

56. The glucose monitor of claim 55 wherein the first and second electrodes are in electrical communication with the collection medium of the first and second collection reservoirs, respectively, and with the power supply through a conductor and the switch.

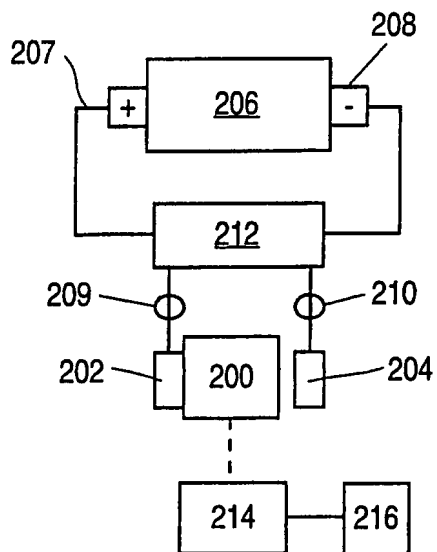
57. The glucose monitor of claim 56 further comprising a collection medium glucose concentration analyzer and calibration means for correlating collection medium glucose concentration with blood glucose concentration.

58. The glucose monitor of claim 55 further comprising a collection medium glucose concentration analyzer and calibration means for correlating collection medium glucose concentration with blood glucose concentration.

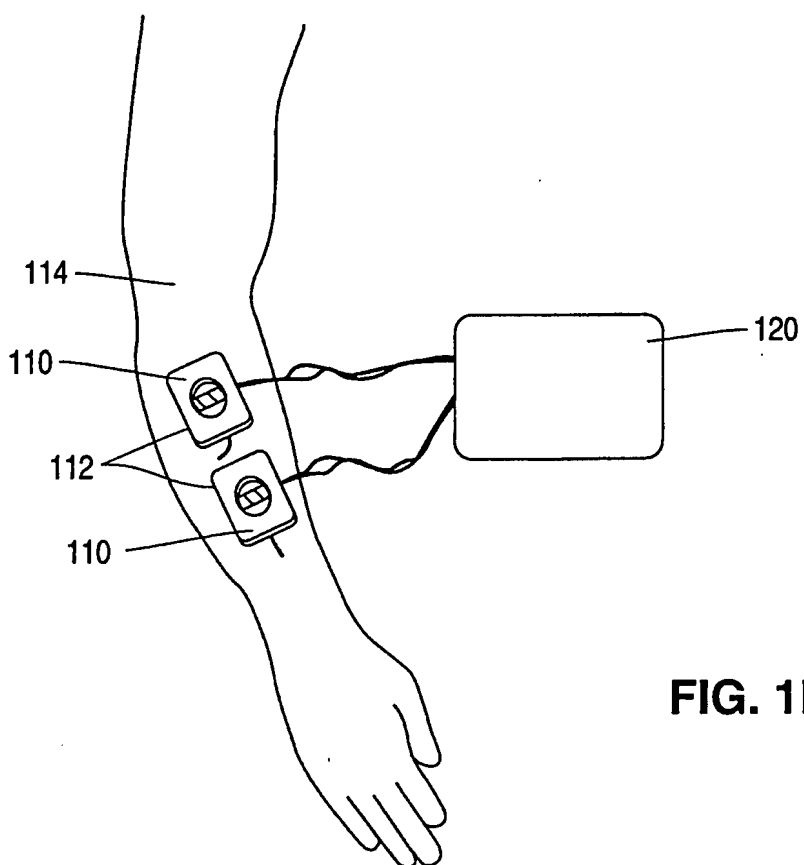
59. The glucose monitor of claim 55 in which the switch is a manual switch.

60. The glucose monitor of claim 55 in which the switch is an automatic programmable switch.

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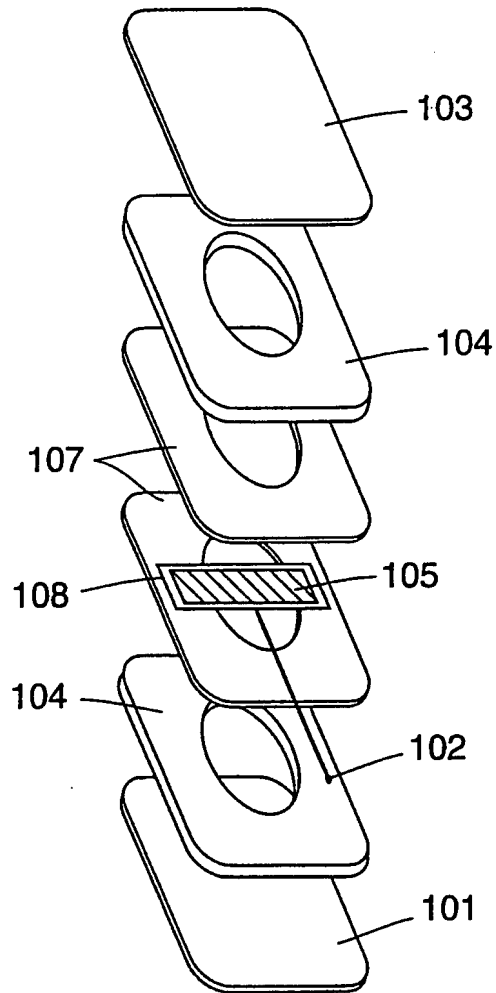
**FIG. 1 A**



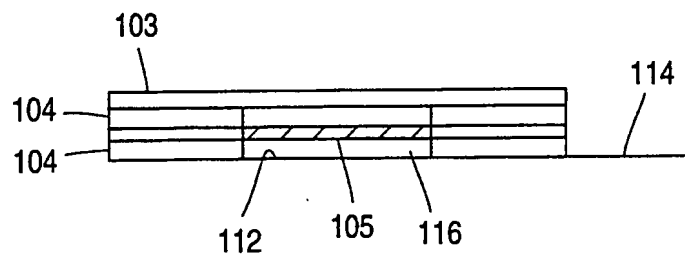
**FIG. 1B**

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**FIG. 1C**



**FIG. 1D**



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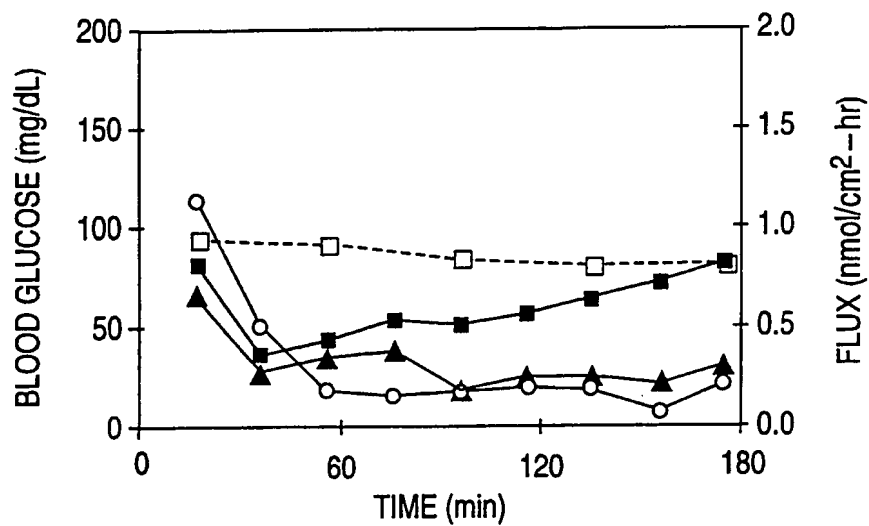


FIG. 2

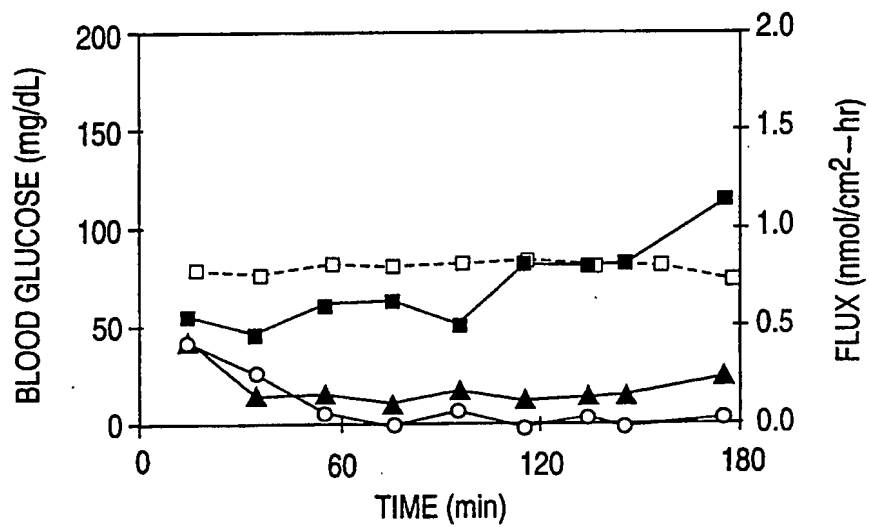


FIG. 3



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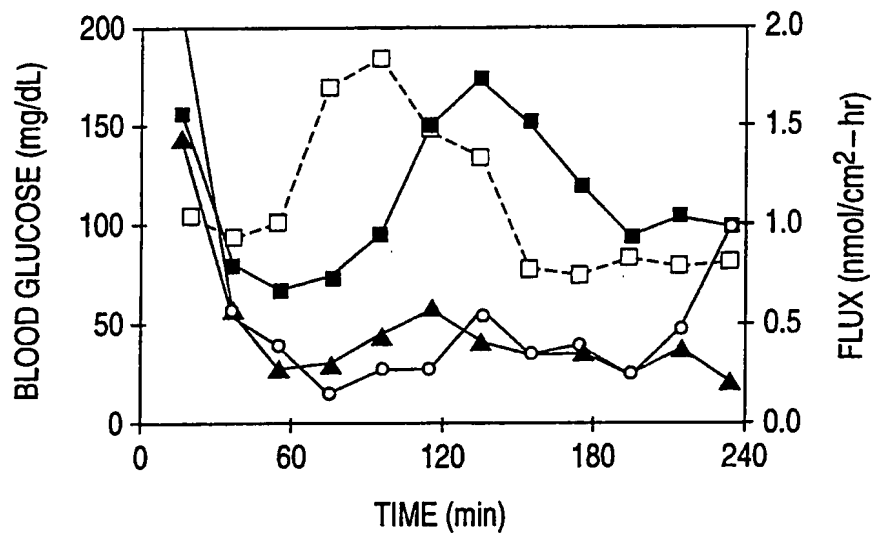


FIG. 4

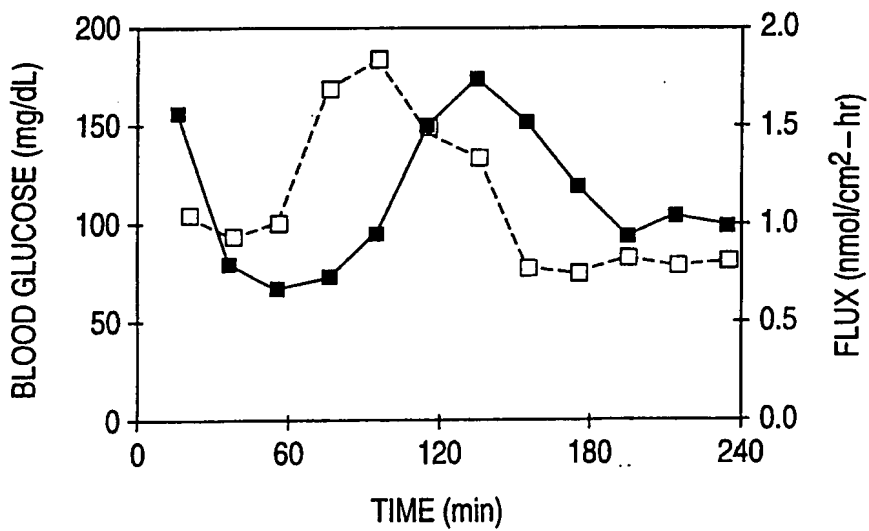


FIG. 5

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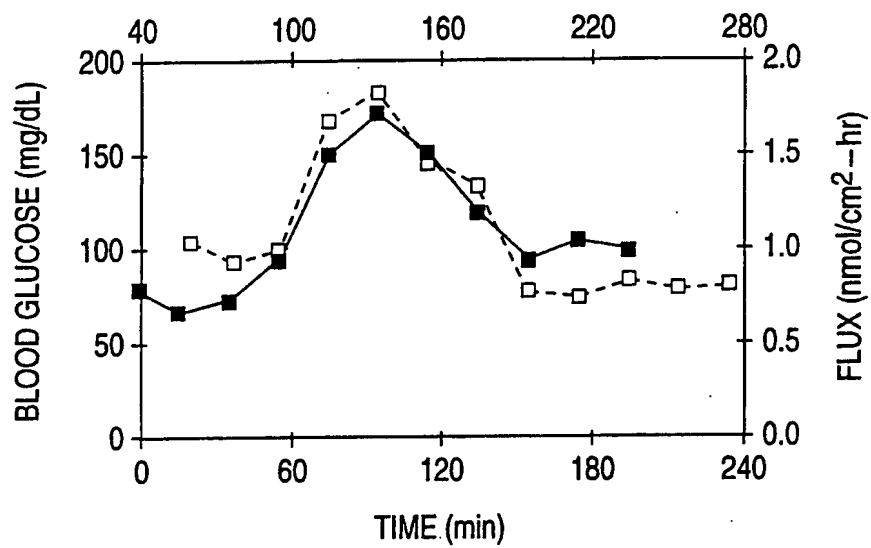


FIG. 6

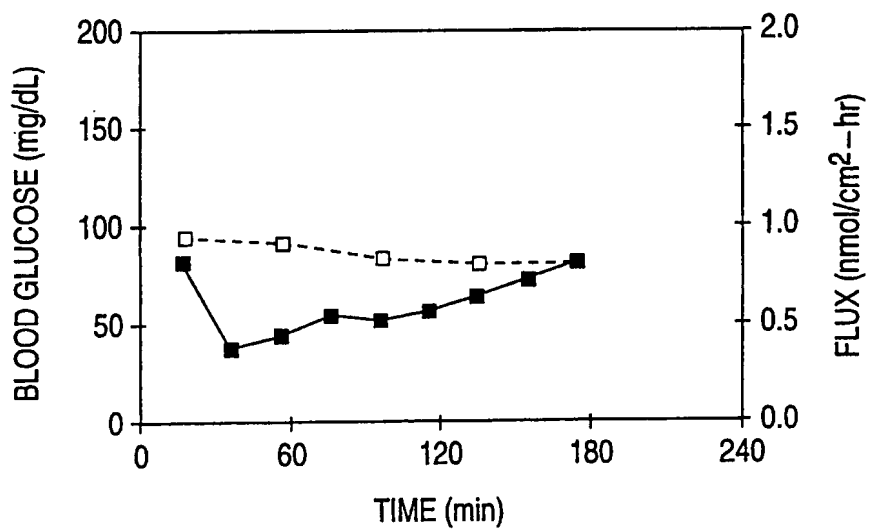


FIG. 7

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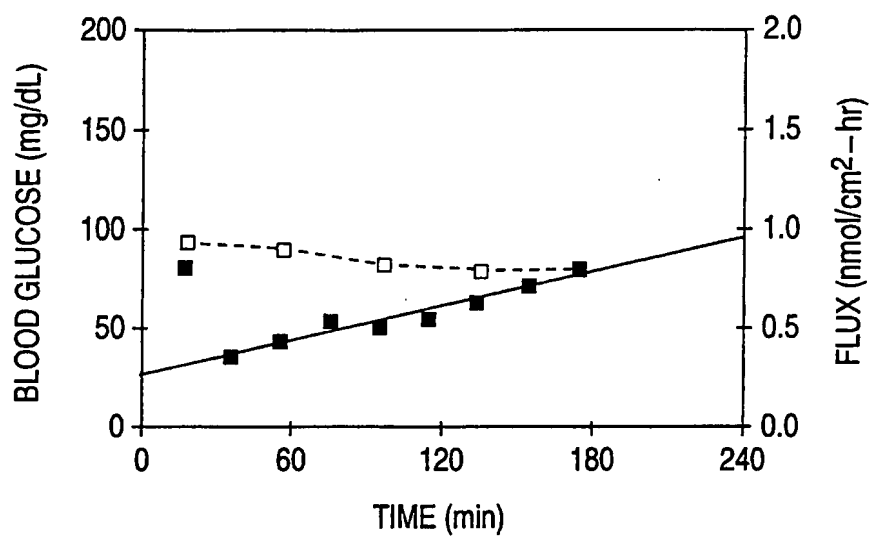


FIG. 8

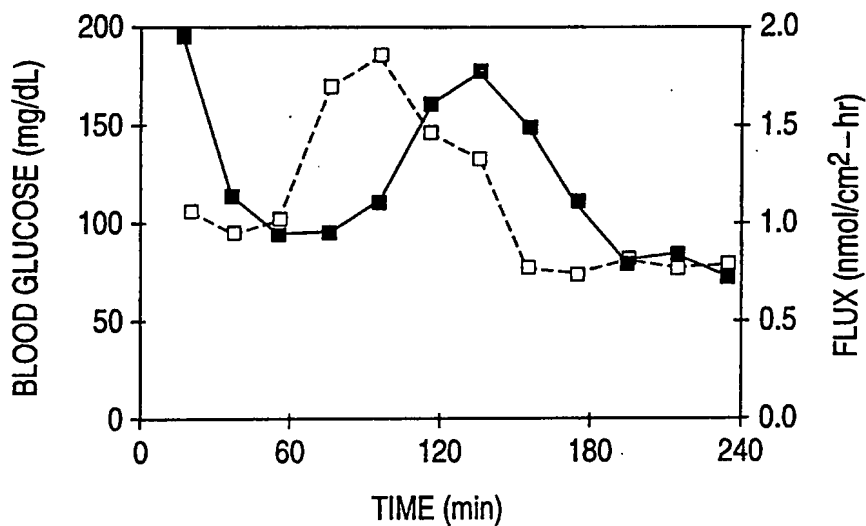


FIG. 9

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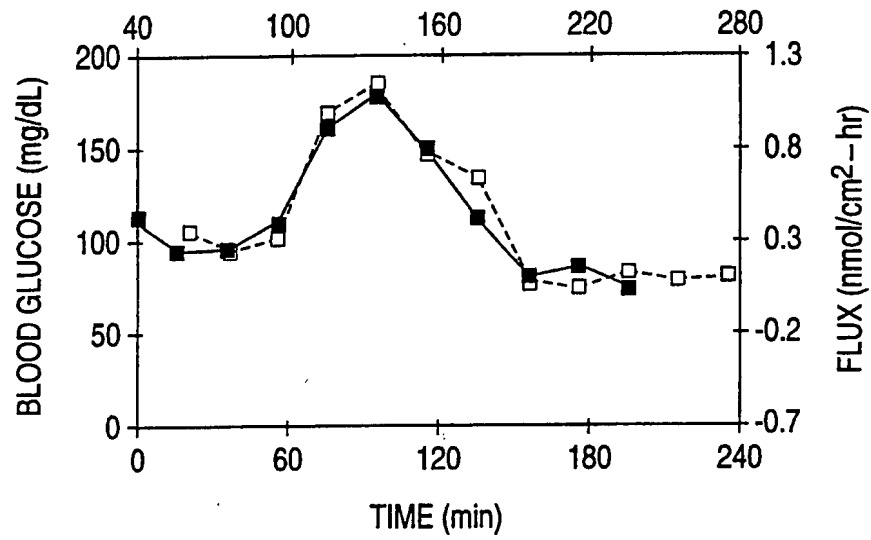


FIG. 10

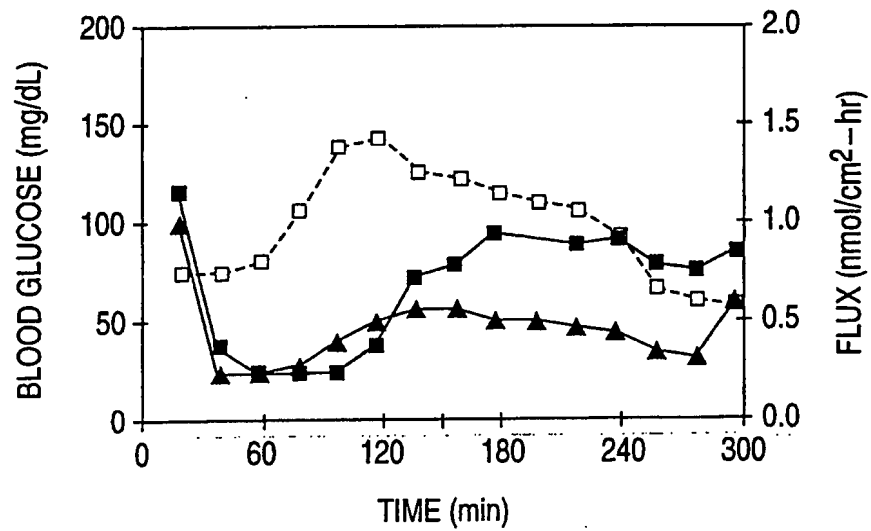


FIG. 11

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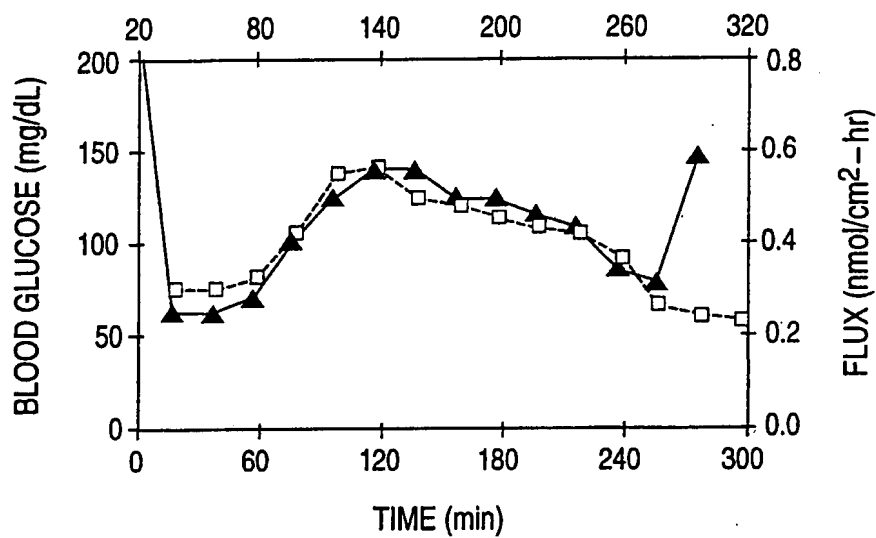


FIG. 12

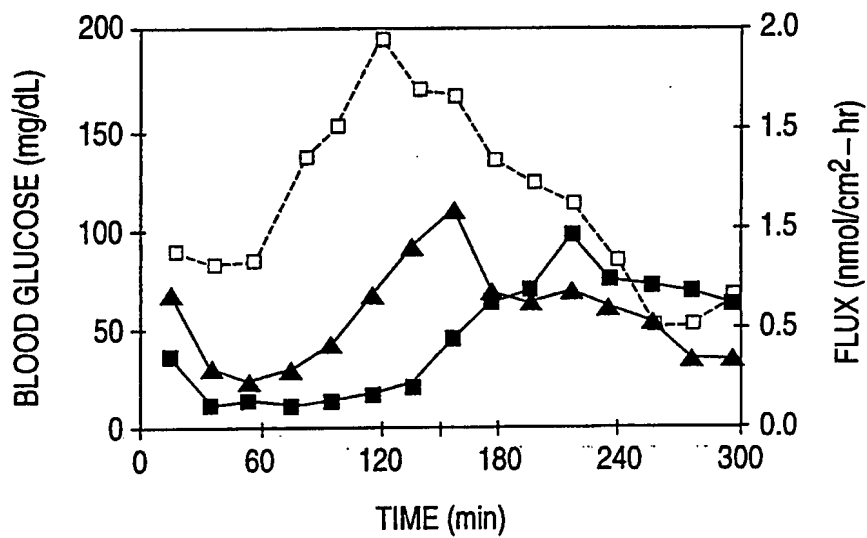


FIG. 13

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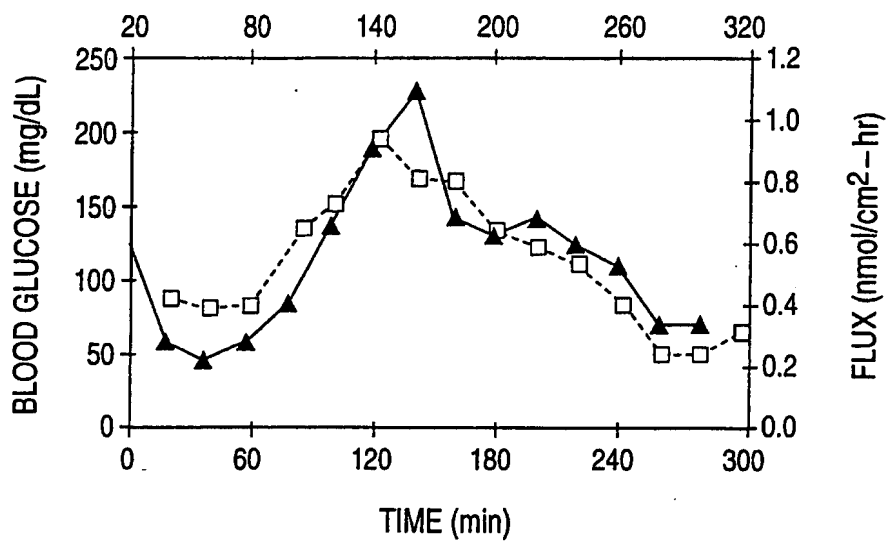


FIG. 14

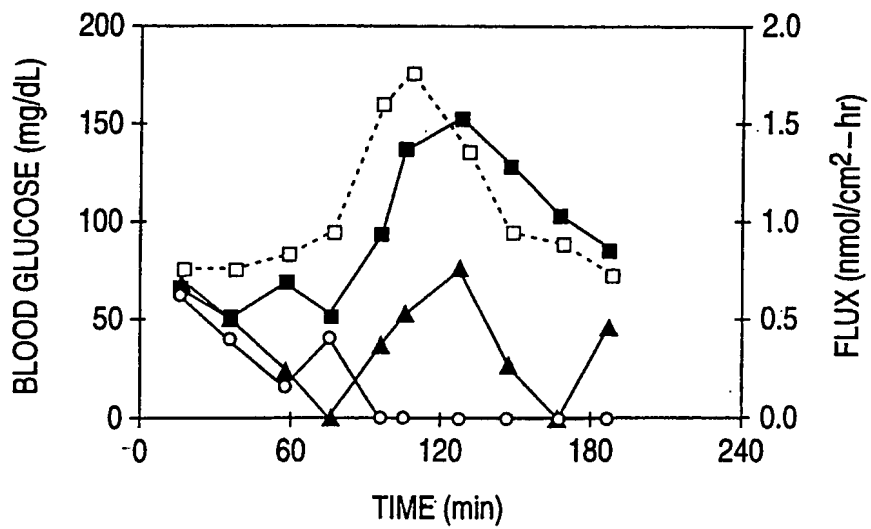


FIG. 15

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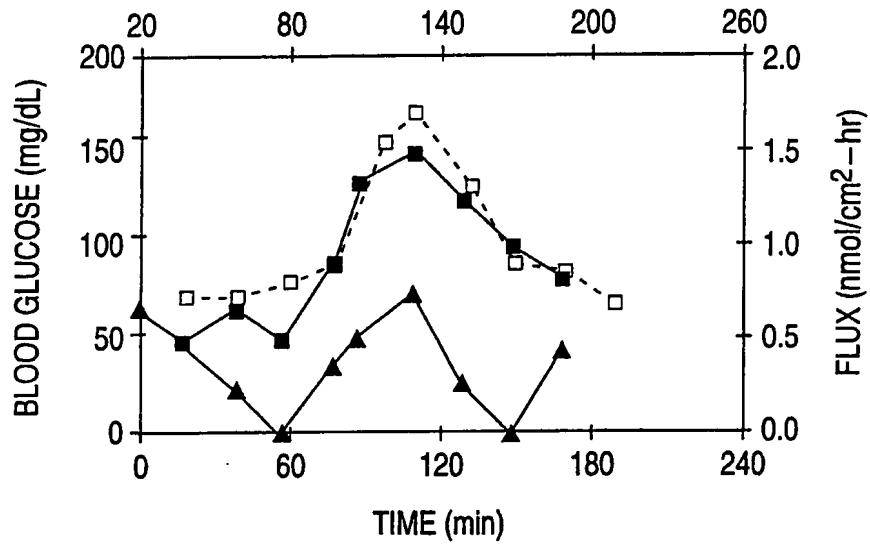


FIG. 16

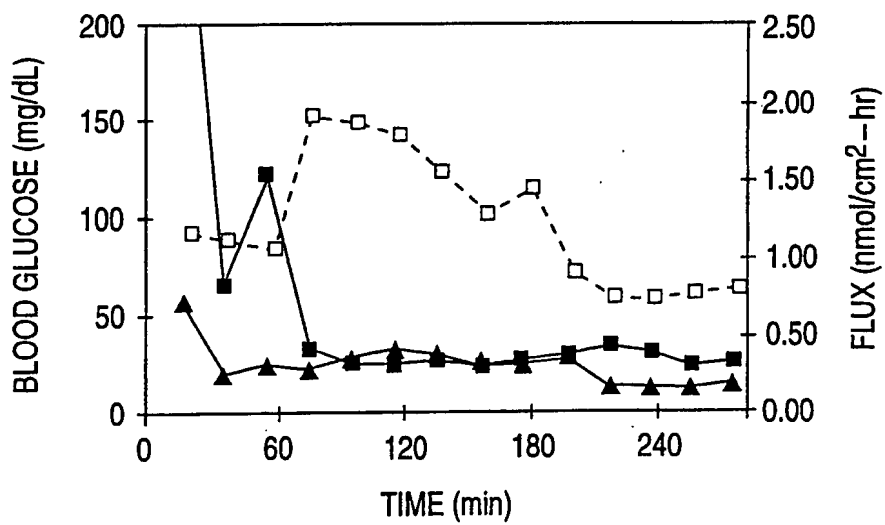


FIG. 17

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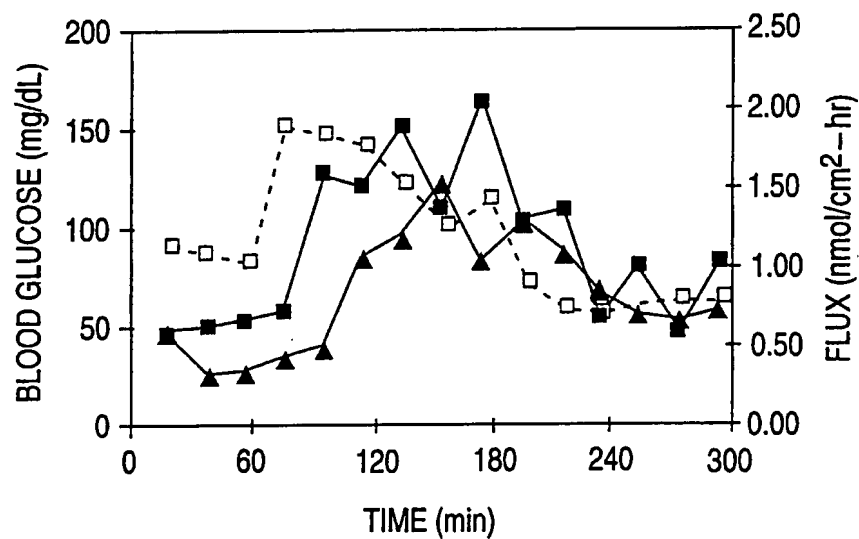


FIG. 18

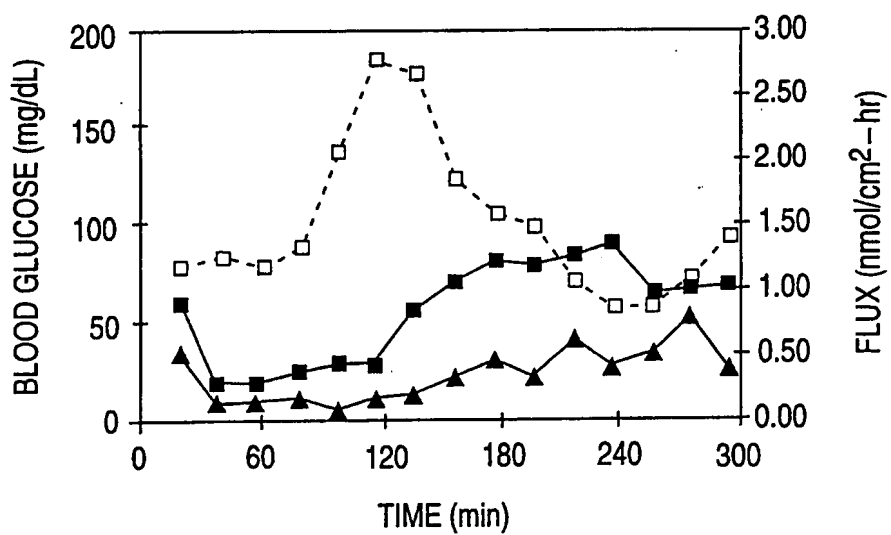


FIG. 19



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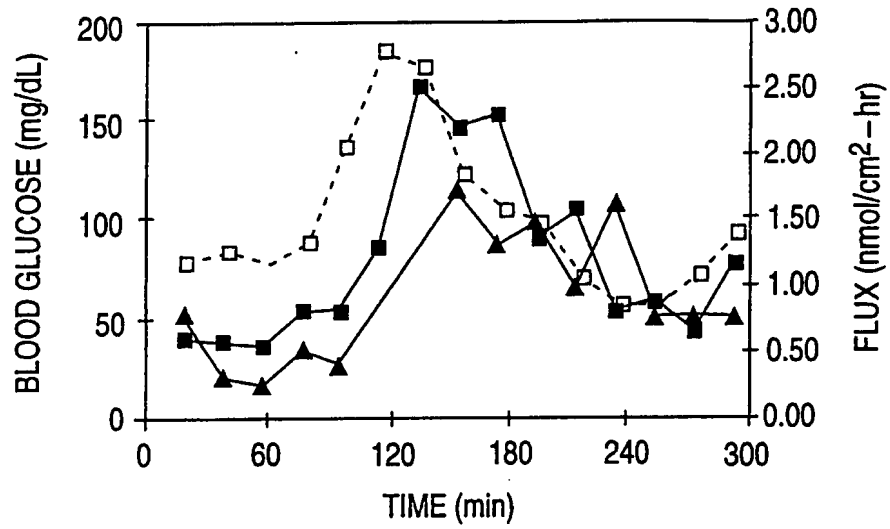


FIG. 20

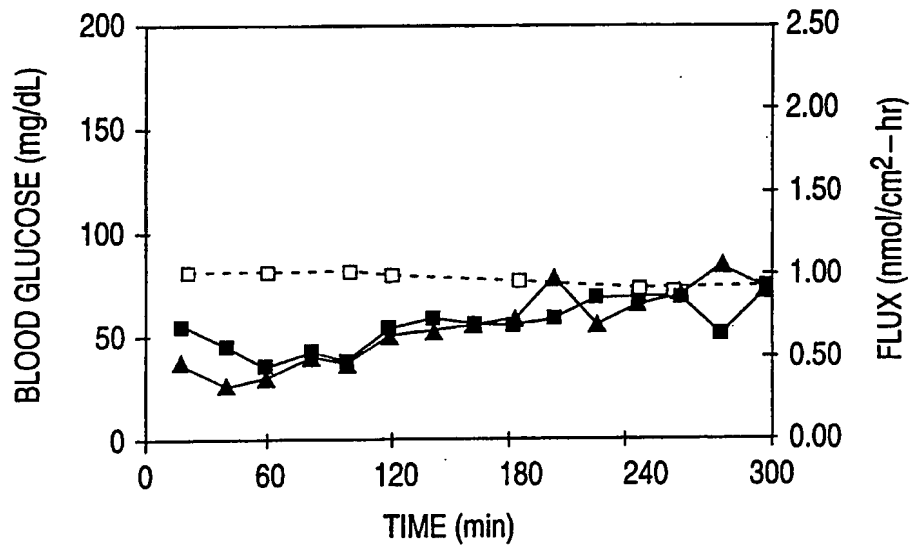
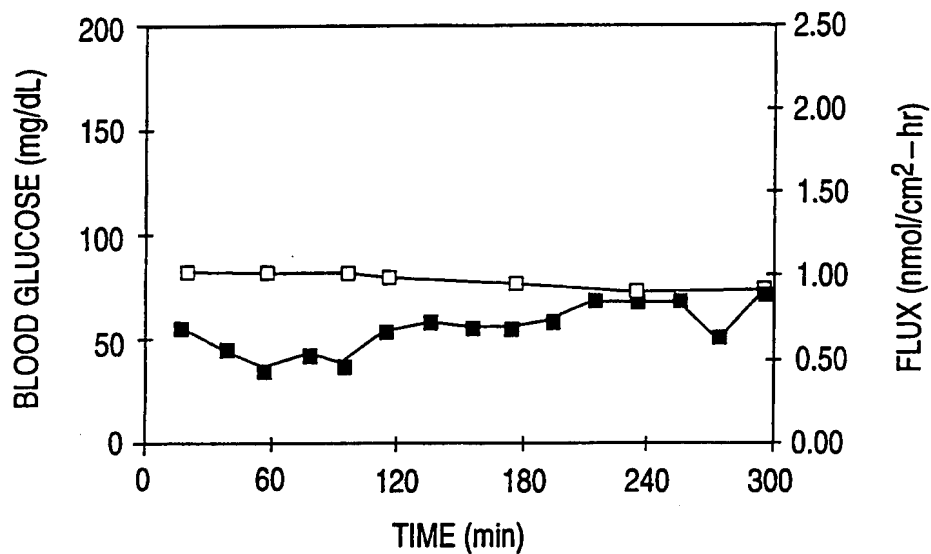
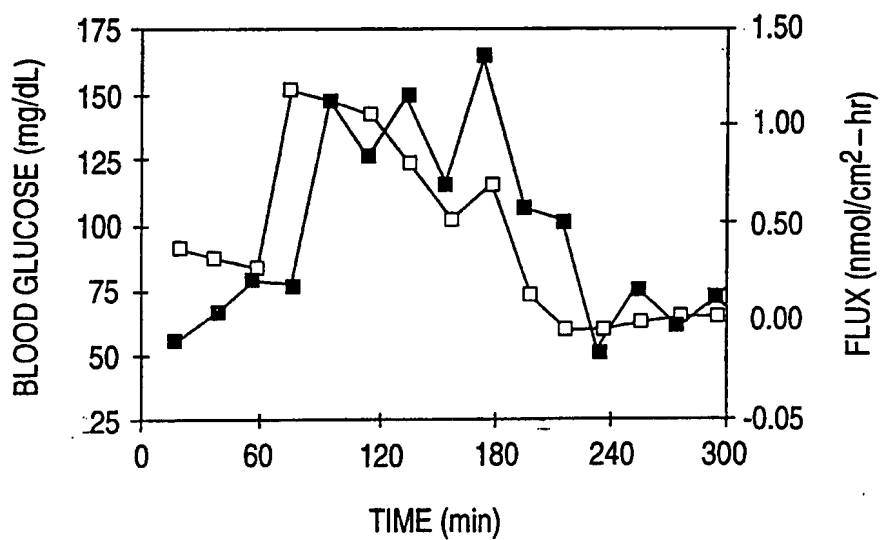


FIG. 21

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**FIG. 22****FIG. 23**

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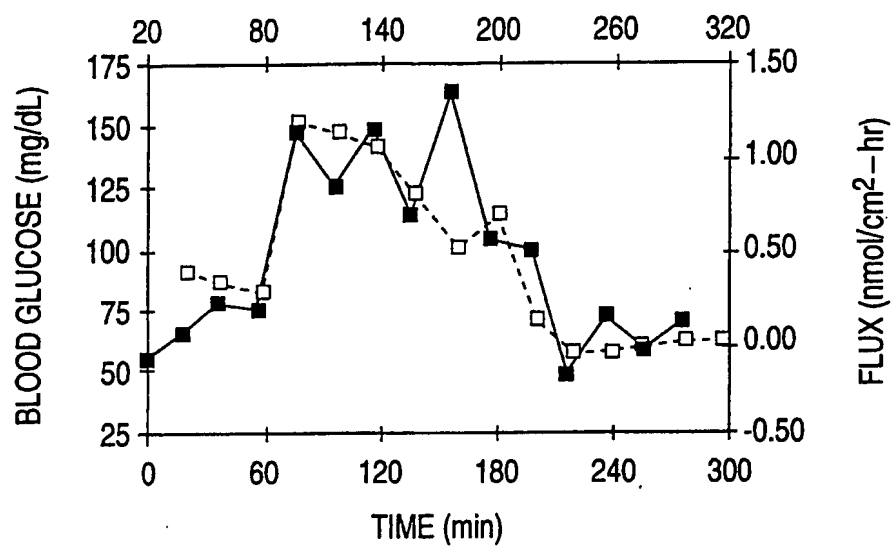


FIG. 24

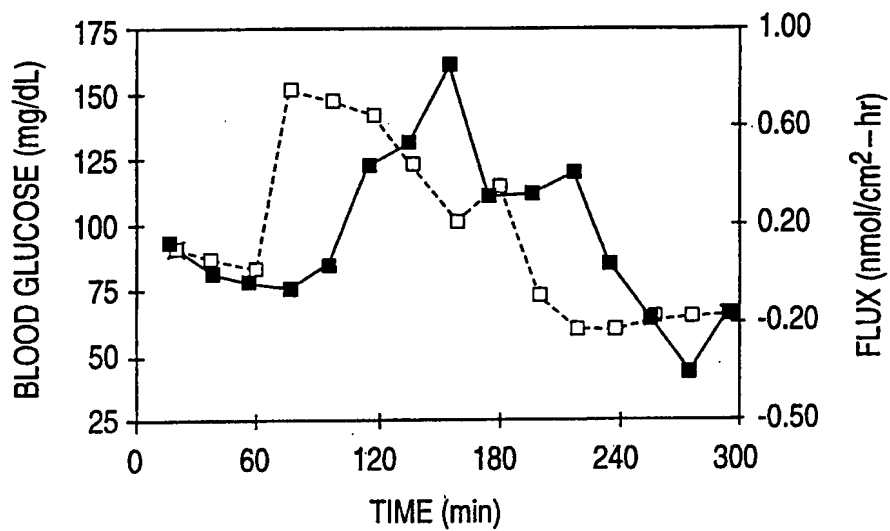


FIG. 25

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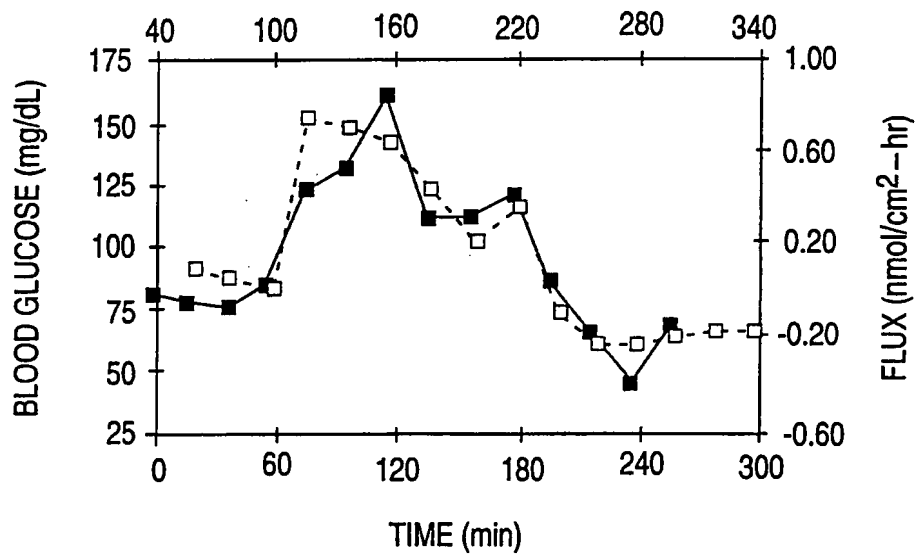


FIG. 26

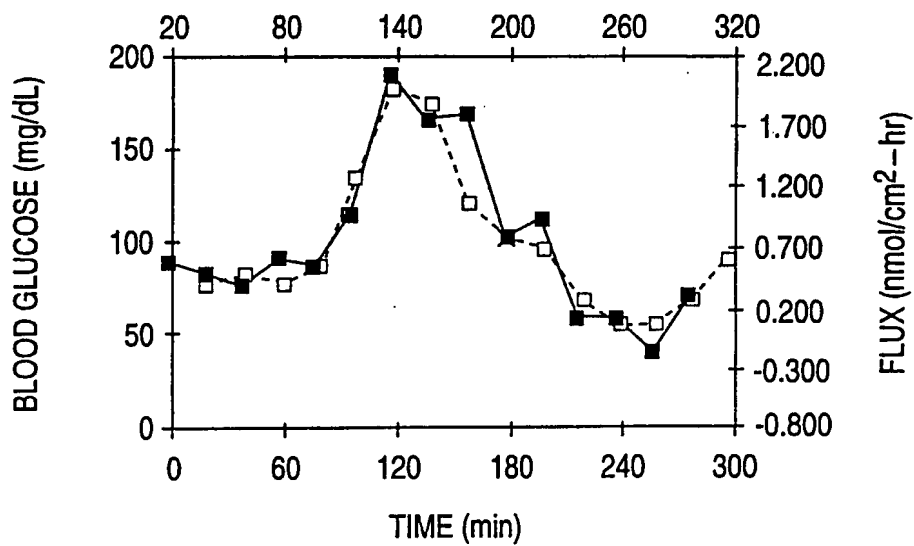


FIG. 27

SUBSTITUTE SHEET (RULE 26)

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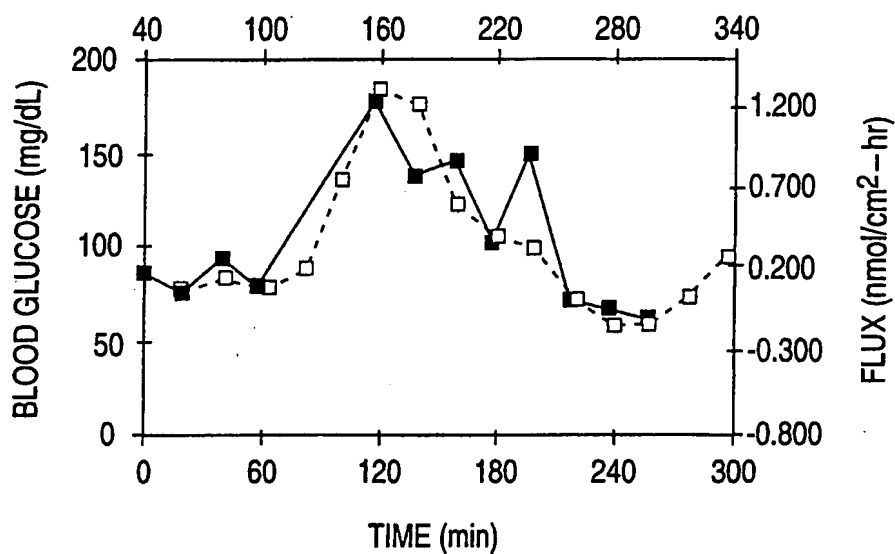


FIG. 28

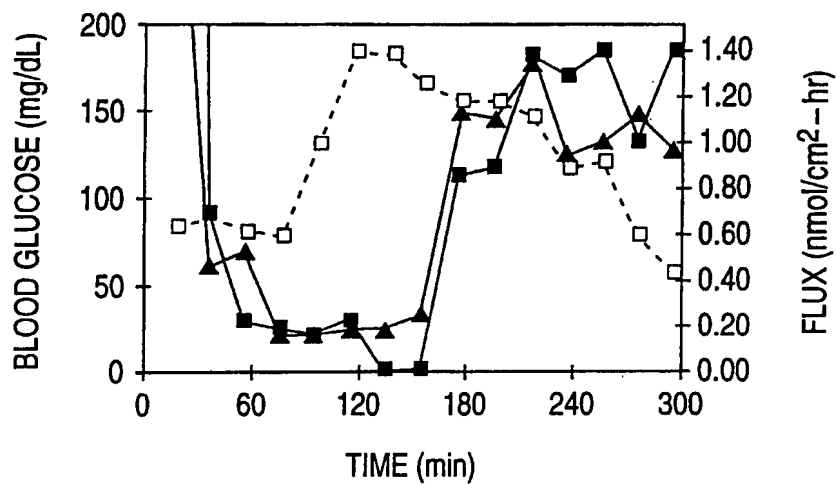


FIG. 29

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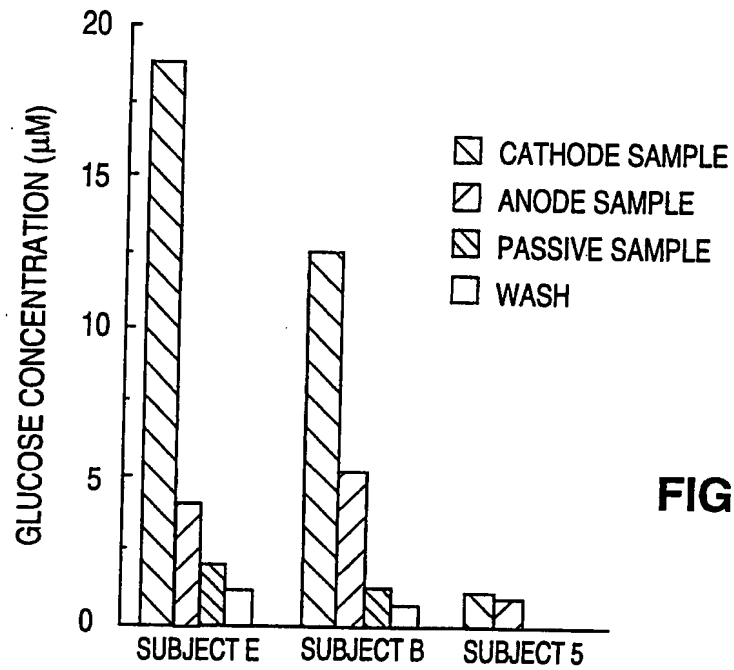


FIG. 30

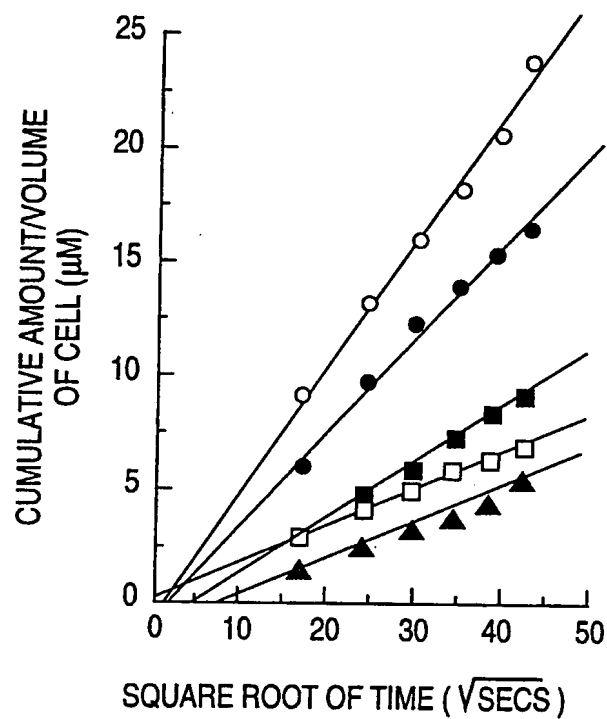


FIG. 31

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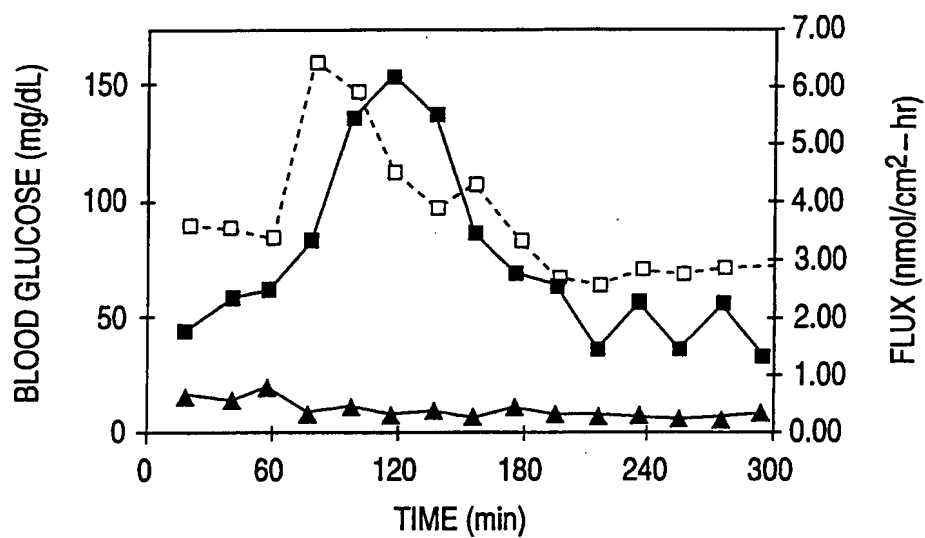


FIG. 32

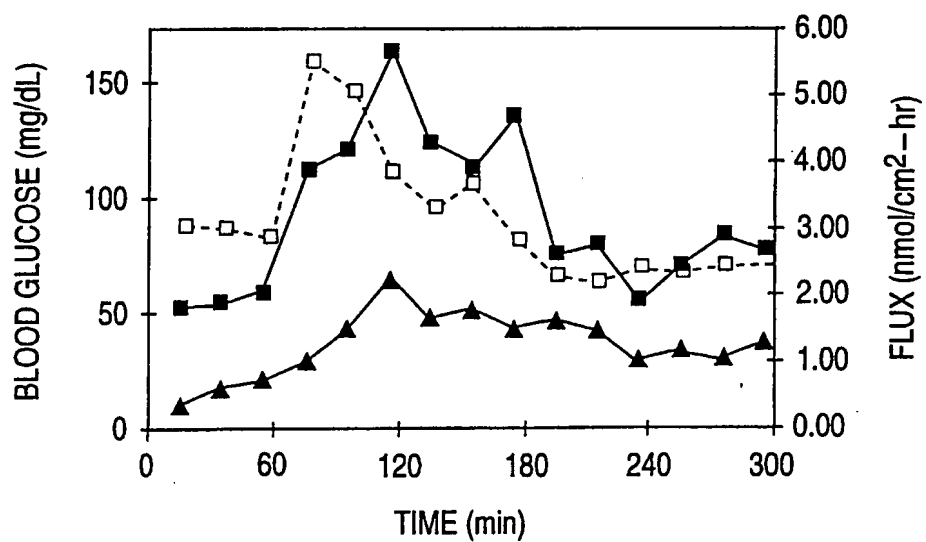


FIG. 33

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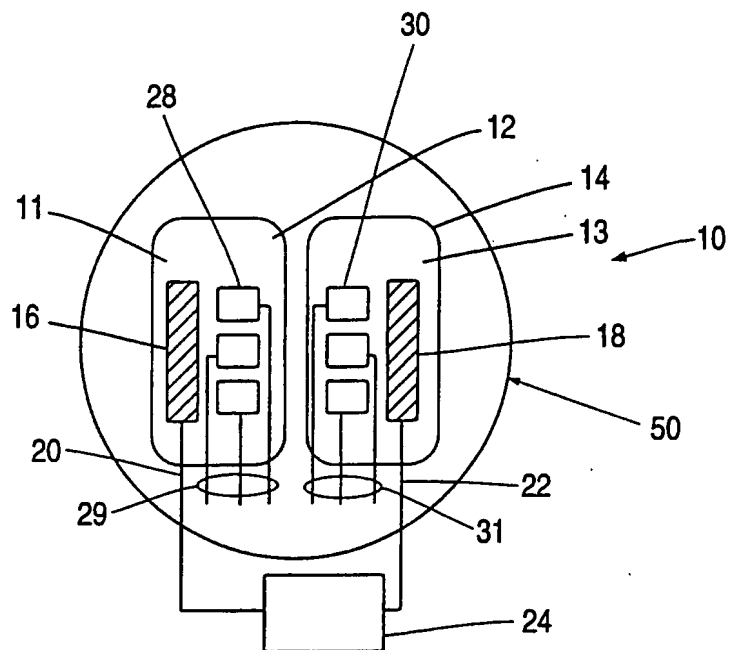


FIG. 34A

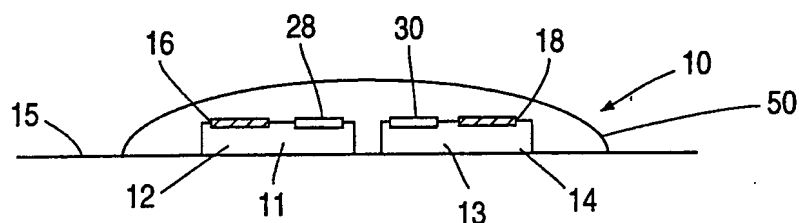
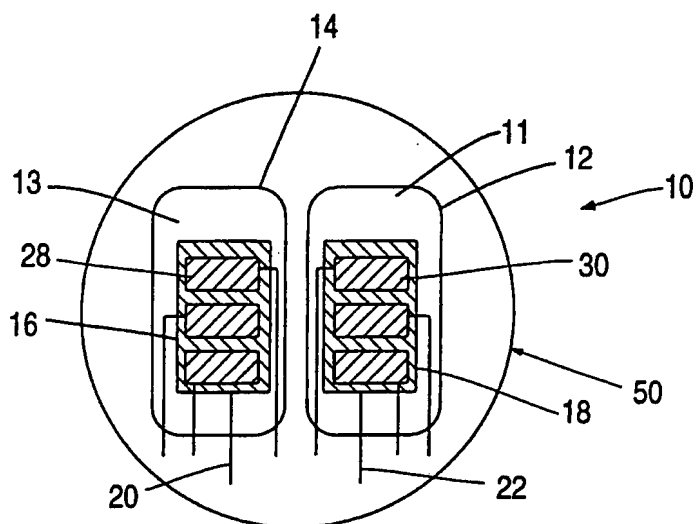


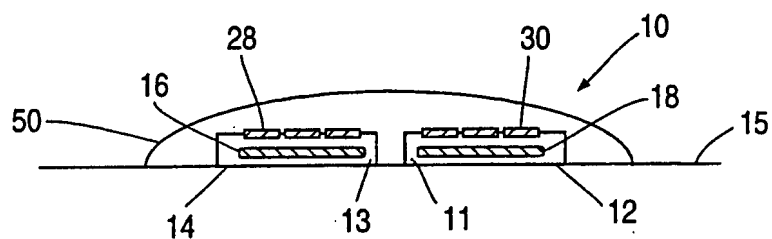
FIG. 34B



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**FIG. 35A**



**FIG. 35B**

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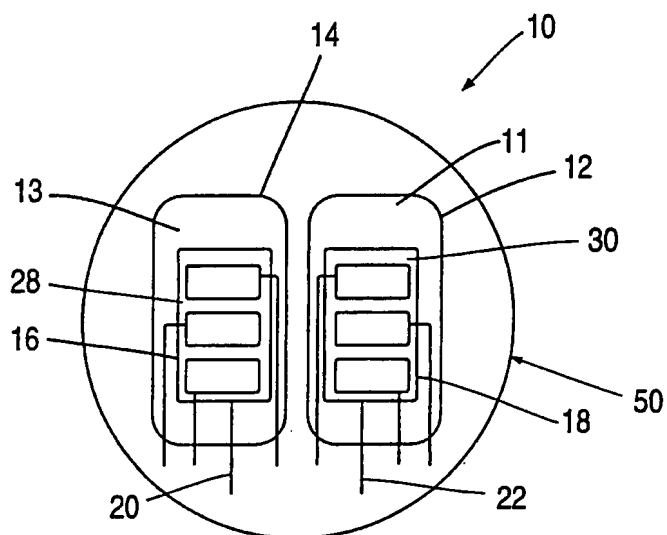


FIG. 36A

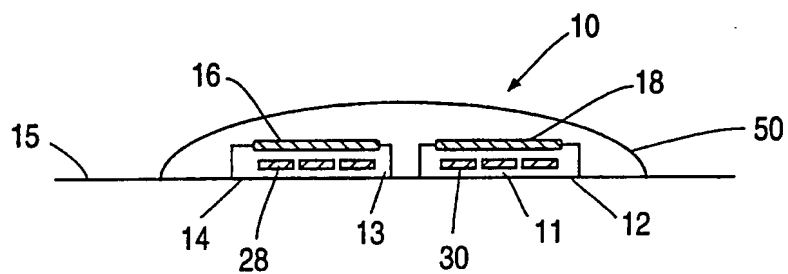


FIG. 36B

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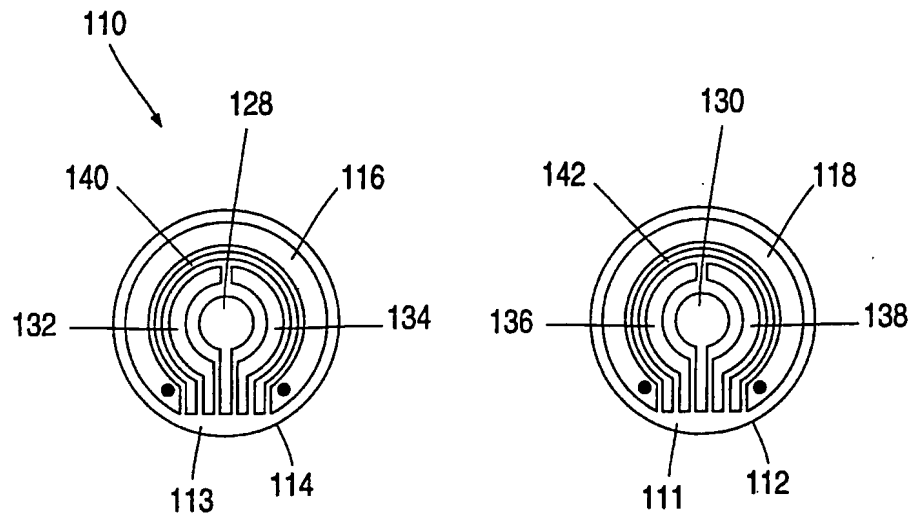


FIG. 37A

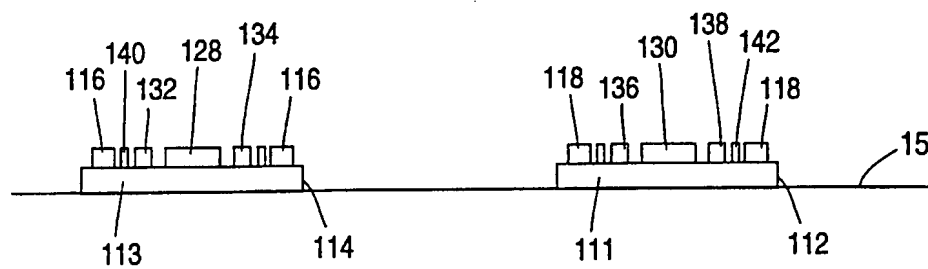
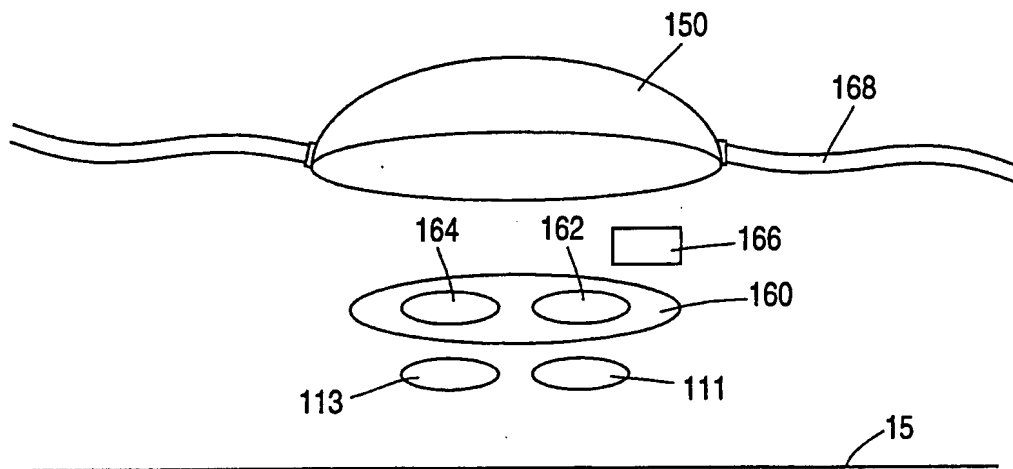
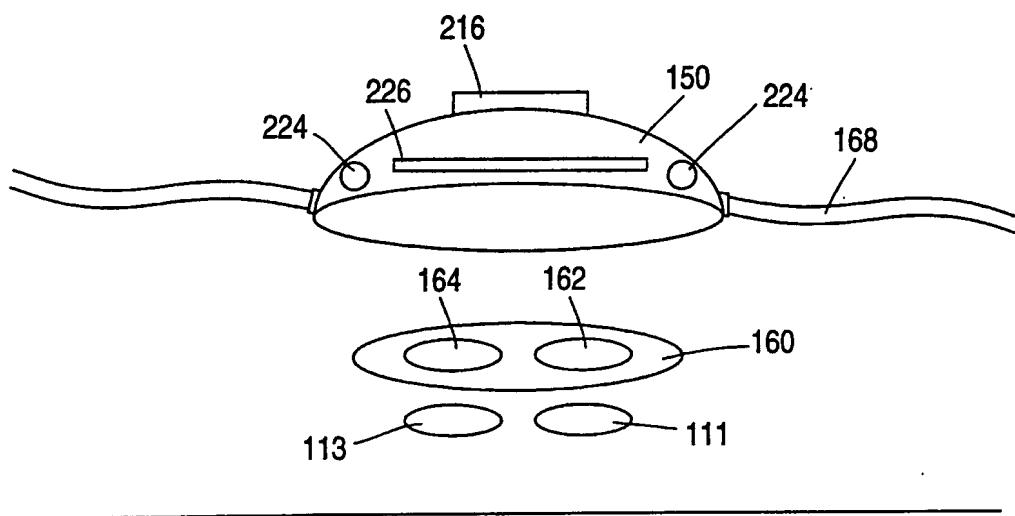


FIG. 37B

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**FIG. 38**



**FIG. 41**

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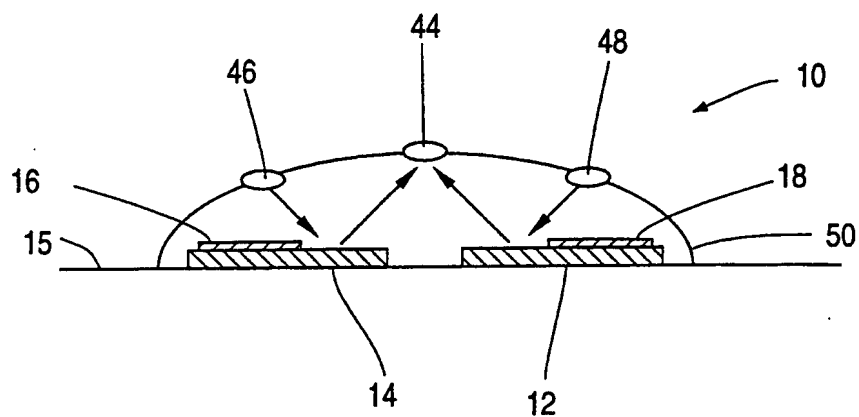


FIG. 39

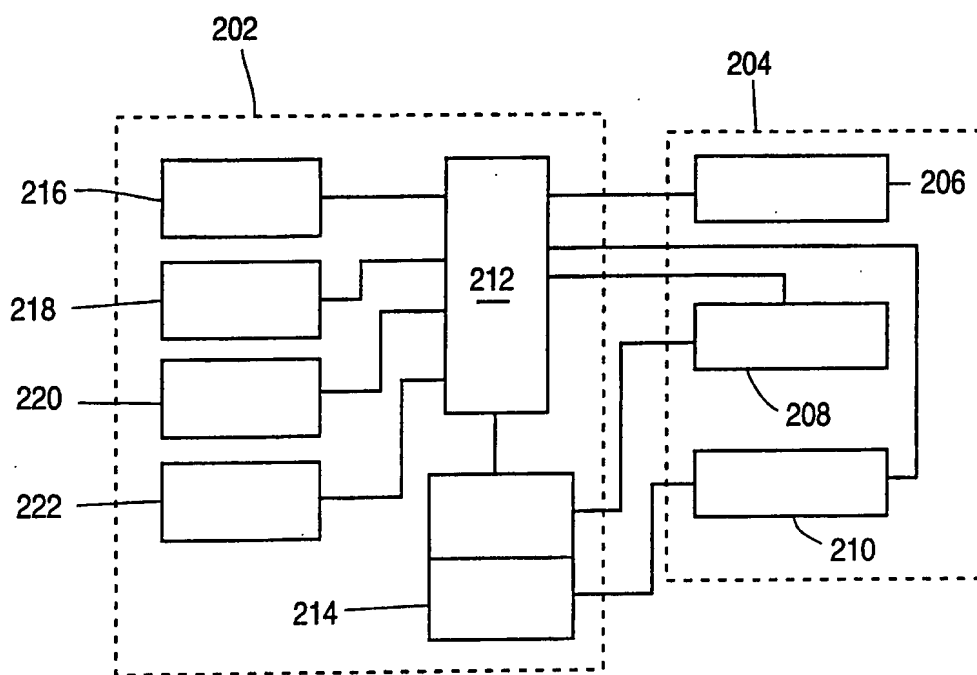


FIG. 40

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/JS 95/07692

## A. CLASSIFICATION OF SUBJECT MATTER

A 61 N 1/30, A 61 B 5/00, A 61 B 10/00

According to International Patent Classification (IPC) or to both national classification and IPC 6

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 N, A 61 B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5 036 861 (W.L. SEMBROWICH et al.) 06 August 1991 (06.08.91), the whole document, especially fig. 6-8; column 2, lines 64-68; column 4, lines 10-52 (cited in the application).	1, 2, 4, 6, 10, 11, 17, 25-30, 32, 33, 36, 39, 40, 42, 43, 48, 55, 56
A	US, A, 5 279 543 (P. GLIKFELD et al.) 18 January 1994 (18.01.94), fig. 2, 3, 7-9B; column 4, lines 22-48; column 6, lines 20-27; column 11, lines 22-24; column 11,	1-5, 11, 25-27, 32-36, 39-42, 45, 48

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search  
11 September 1995

Date of mailing of the international search report

30.10.95

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Authorized officer

LUDWIG e.h.

# INTERNATIONAL SEARCH REPORT

Intern. Application No.  
PCT/US 95/07692

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	<p>line 32 - column 12, line 31; column 18, lines 4-6; claims 29-32 (cited in the application). --</p>	
A	<p>EP, A, 0 483 883 (R. TAPPER) 06 May 1992 (06.05.92), abstract; page 6, lines 51-53; page 7, lines 20-28, 45-54. --</p>	<p>37,54, 55,59, 60</p>
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## ANHANG

zum internationalen Recherchen-  
bericht über die internationale  
Patentanmeldung Nr.

## ANNEX

to the International Search  
Report to the International Patent  
Application No.

## ANNEXE

au rapport de recherche inter-  
national relatif à la demande de brevet  
international n°

PCT/US 95/07692 SAE 113110

In diesem Anhang sind die Mitglieder  
der Patentfamilien der im obenge-  
nannten internationalen Recherchenbericht  
angeführten Patentdokumente angegeben.  
Diese Angaben dienen nur zur Unter-  
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This Annex lists the patent family  
members relating to the patent documents  
cited in the above-mentioned inter-  
national search report. The Office is  
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La présente annexe indique les  
membres de la famille de brevets  
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dans le rapport de recherche inter-  
national visée ci-dessus. Les renseigne-  
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